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CONTENTS

| | |
|---|----|
| The Chemical Composition of Florida Everglades Peat Soils, with Special Reference to Their Inorganic Constituents. HARALD EDWIN HAMMAR..... | 1 |
| Variations in the Calcium and Magnesium Contents of Pea Plants on Different Soil Types. J. F. FONDER..... | 2 |
| The Ultimate Natural Structure of Soils. GEORGE JOHN BOUYOUKOS..... | 27 |
| Soil Profile Studies: I. Soil as an Independent Body and Soil Morphology. I. S. JOFFE..... | 30 |
| Composition of Natural Organic Materials and Their Decomposition in the Soil: IV. The Nature and Rapidity of Decomposition of the Various Organic Complexes in Different Plant Materials, Under Aerobic Conditions. FLORENCE G. TENNEY AND SELMAN A. WAKSMAN..... | 31 |
| The Availability of Nitrogenous Fertilizers to Rice. R. P. BARTHOLOMEW..... | 34 |
| Soil Type and Crop Adaptation. J. H. STALLINGS..... | 37 |
| The Use of Dyes in the Isolation of a Nitrite Oxidizing Organism. CHAS. C. PROUTY..... | 38 |
| The Vertical Distribution of Soil Bases and Acidity in Some Illinois Soils. HERBERT LUNT..... | 39 |
| First Subcommittee of the Fifth Commission, International Society of Soil Science Meets at Danzig. HUGO CARL..... | 42 |
| The Laws of Soil Colloidal Behavior: I. SANTE MATTSON..... | 47 |
| A Study of Diastase Activity in Plants: The Effect of Phosphates in the Soil Media. D. T. ENGLISH AND LOUIS GERBER..... | 52 |
| A Method for the Determination of Total Carbon and Also for the Estimation of Carbon Dioxide Evolved from Soils. A. FLOYD HECK..... | 53 |
| Colloidal Properties of Willamette Valley Soils. R. E. STEPHENSON..... | 57 |
| The Fermentation of Glucose and Xylose by the Nodule Bacteria from Alfalfa, Clover, Pea, and Soybean. MARIAN FOOTE, W. H. PETERSON, AND E. B. FRED..... | 58 |
| Book Reviews..... | 60 |
| Relation of Calcium to the Nodulation of Soybeans on Acid and Neutral Soils. ALBRECHT AND FRANKLIN L. DAVIS..... | 62 |
| Decomposition of Citric Acid by Soil. L. A. DEAN AND A. L. DEAN..... | 63 |
| The Relation Between the Absorbed and the Exchangeable Calcium and Magnesium Content of a Soil Four Years After Additions. W. H. MACINTIRE AND K. B. SANDERS..... | 64 |
| The Use of Bacteriostatic Dyes in the Isolation of <i>Rhizobium leguminosarum</i> Frank. IVAN A. ANDERSON..... | 65 |
| Contribution to the Chemical Composition of Peat: V. The Role of Microorganisms in Peat Formation and Decomposition. SELMAN A. WAKSMAN AND KENNETH R. STEVENS..... | 66 |
| The Microflora of Leached Alkali Soils: I. Synthetic Alkali Soil. J. DUNLEY GREAVES..... | 67 |
| Composition and Nitrification Studies on <i>Crotalaria Striata</i> . W. A. LEUKEL, M. BARNETTE AND J. B. HESTER..... | 68 |
| The Laws of Soil Colloidal Behavior: II. Cataphoresis, Flocculation, and Dispersion. SANTE MATTSON..... | 69 |

| | |
|--|-----|
| ✓ Extraction of Adsorbed Cations from Soil by Electrodialysis. BENJAMIN D. WILSON.. | 411 |
| The Value of Raw Sewage Sludge as Fertilizer. J. F. MULLER..... | 423 |
| Studies of Nitrogen Fixation by the Root Nodule Bacteria of the Leguminosae. E. W. HOPKINS..... | 433 |
| Nitrate Changes in a Fertile Soil as Influenced by Sodium Nitrate and Ammonium Sulfate. NANDOR FORGES..... | 449 |
| Diurnal, Average, and Seasonal Soil Temperature Changes at Davis, California. ALFRED SMITH..... | 457 |
| ✓ Soil Profile Studies: II. Methods Used in the Profile Survey of New Jersey Soils. J. S. JOFFE AND L. L. LEE..... | 469 |

ILLUSTRATIONS

PLATES

CHEMICAL COMPOSITION OF FLORIDA EVERGLADES PEAT SOILS, WITH SPECIAL REFERENCE TO THEIR INORGANIC CONSTITUENTS

Relative Amounts of Parent Plant Material in Everglades Soils.....

SOIL TYPE AND CROP ADAPTATION

| | |
|--|-----|
| Grape Plants Set March 4, 1927, Photographed July 21, 1927..... | 111 |
| Fig. 1. Grapes on Blanton fine sand..... | 111 |
| 2. Grapes on Leon fine sand (loamy phase)..... | 111 |
| Plants Set in Early March, 1927, Photographed July 13, 1927..... | 113 |
| Fig. 1. Peaches, pears, and plums on Blanton fine sand..... | 113 |
| 2. Pecans on Leon fine sand (loamy phase)..... | 113 |
| Blueberries; Foreground Leon Fine Sand; Background Blanton Fine Sand.... | 115 |
| Fig. 1. Tung Oil trees; left, Norfolk fine sand; right, Leon fine sand. Plants all set at the same time and cultivated uniformly throughout..... | 117 |
| 2. Crotalaria on Leon fine sand. Photographed November 2, 1927..... | 117 |
| Fig. 1. Corn on Portsmouth Loamy fine sand. Photographed July 13, 1927. | 119 |
| 2. Corn on Leon fine sand. Photographed July 13, 1927..... | 119 |
| Fig. 1. Peppers on St. John's loamy fine sand. Plants set in April, 1927, photographed July 13, 1927..... | 121 |
| 2. Japanese sugar cane on Portsmouth loamy fine sand. Photographed November 5, 1927, produced 40 tons to the acre..... | 121 |
| Fig. 1. Sweet potatoes grown on Leon fine sand, loamy phase. All from one hill..... | 123 |
| 2. Peppermint; foreground, Cypress Pond soil; left background, Leon fine sand, loamy phase. Photographed July 13, 1927..... | 123 |

MOD FOR THE DETERMINATION OF TOTAL CARBON AND ALSO FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS

Set-Up Used in Carbon Dioxide Evolution from Soils..... 233

EFFECT OF CALCIUM TO THE NODULATION OF SOYBEANS ON ACID AND NEUTRAL SOILS

| | |
|---|-----|
| Soybean Nodules by Inoculation with Bacteria in Colloidal Clay..... | 275 |
| Fig. 1. Distilled water suspension..... | 275 |
| 2. Calcium chloride supernatant..... | 275 |
| 3. Calcium chloride flocculent..... | 275 |
| 4. Colloidal clay suspension..... | 275 |
| 5. Potassium chloride supernatant..... | 275 |
| 6. Potassium chloride flocculent..... | 275 |
| Difference in Coloration and Nodulation of Soybeans Due to Calcium Treatment..... | 277 |
| Fig. 1. Differences in color in consequence of calcium treatment (Marion Silt Loam). Left, cultures and calcium chloride. Right, cultures only. | 277 |

2. Nodulation differences in consequence of calcium treatment. (Marion Silt Loam). Above, cultures and calcium. Below, cultures only... 277
- Plate 3. Soybean Growth on Putnam Silt Loam as Influenced by Calcium Treatments. 279

DECOMPOSITION OF CITRIC ACID BY SOIL

- Plate 1. Cowpeas..... 287

SOIL PROFILE STUDIES: II. METHODS USED IN THE PROFILE SURVEY OF NEW JERSEY SOILS

- Plate 1. Tube Used for Sampling Soils for Volume-Weight Determinations..... 479

TEXT FIGURES

THE CHEMICAL COMPOSITION OF FLORIDA EVERGLADES PEAT SOILS, WITH SPECIAL REFERENCE TO THEIR INORGANIC CONSTITUENTS

- Fig. 1. Location of Soils Used in This Investigation..... 4

COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL:

IV. THE NATURE AND RAPIDITY OF DECOMPOSITION OF THE VARIOUS ORGANIC COMPLEXES IN DIFFERENT PLANT MATERIALS, UNDER AEROBIC CONDITIONS

- Fig. 1. Decomposition of Various Chemical Constituents of Corn Stalks without Additional Nutrient Salts..... 63
2. Decomposition of Various Chemical Constituents of Corn Stalks with Additional Nutrient Salts..... 64
3. Decomposition of Various Chemical Constituents of Rye Straw without Additional Nutrient Salts..... 69
4. Decomposition of Various Chemical Constituents of Rye Straw with Additional Nutrient Salts..... 70
5. Decomposition of Various Chemical Constituents of Oak Leaves without Additional Nutrient Salts..... 74
6. Decomposition of Various Chemical Constituents of Oak Leaves with Additional Nutrient Salts..... 74
7. Decomposition of Various Chemical Constituents of Alfalfa without Additional Nutrient Salts..... 78
8. Decomposition of Various Chemical Constituents of Alfalfa with Additional Nutrient Salts..... 79

THE VERTICAL DISTRIBUTION OF SOIL BASES AND ACIDITY IN SOME ILLINOIS SOILS

- Fig. 1. Map Showing Location of Experiment Fields from Which Samples Were Taken..... 138
2. Buffer Curves of the A₁ Horizon..... 147
3. Graphic Presentation of Data from Untreated Plots of the Ewing, Toledo and Unionville Fields..... 146
4. Graphic Presentation of Data from Untreated Plots of the Joliet, Mt. Morris, and Urbana Fields..... 150
5. Graphic Presentation of Data from Untreated Plots of the Hartsburg and Oquawka Fields..... 151
6. Effects of Treatments on H-Ion Concentration (Expressed as pH) of Soils from Four Fields..... 153
7. Effect of Treatments on H-Ion Concentration (Expressed as pH) of Soils from Four Fields..... 154
8. Effect of Treatments on H-Ion Concentration (Expressed as pH)..... 154

| | |
|--|-----|
| 9. Effects of Treatments upon the Soil Lime Requirement as Measured by the Hutchinson-MacLennan and the Hopkins Methods in Grams CaCO_3 per 100 Gm. Soil..... | 160 |
| 10. Effect of Treatments upon the Soil Lime Requirement as Measured by the Hutchinson-MacLennan and the Hopkins Methods, in Grams CaCO_3 per 100 Gm..... | 161 |
| 11. Effect of Treatments upon the Soil Lime Requirement as Measured by the Hutchinson-MacLennan and the Hopkins Methods, in Grams CaCO_3 per 100 Gm..... | 162 |
| 12. Effect of Treatments upon the Soil Lime Requirement as Measured by the Hutchinson-MacLennan Method, in Grams CaCO_3 per 100 Gm. Soil..... | 163 |
| 13. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil..... | 167 |
| 14. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil..... | 169 |
| 15. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil..... | 171 |
| 16. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil..... | 172 |
| 17. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil..... | 173 |

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: I

| | |
|--|-----|
| Fig. 1. The Variations in the Values of x , y , z , and e in the Donnan Distribution of Ions in a Bentonite Gel and in the Outside Solution..... | 198 |
| 2. The Influence of the Valence of the Anion on the Suppression of the P. D., the Swelling, and the Viscosity of Bentonite..... | 200 |
| 3. The Influence of NaOH on the P. D., the Swelling and the Viscosity of Electro-dialyzed Bentonite..... | 216 |

A METHOD FOR THE DETERMINATION OF TOTAL CARBON AND ALSO FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS

| | |
|--|-----|
| Fig. 1. Apparatus, Including a New Carbon Dioxide Trap, for the Determination of Total Carbon by Wet Combustion..... | 226 |
| 2. Apparatus for the Estimation of Carbon Dioxide Evolved from a Soil..... | 229 |
| 3. Rate of Evolution of Carbon Dioxide from Various Substances..... | 230 |

THE FERMENTATION OF GLUCOSE AND XYLOSE BY THE NODULE BACTERIA FROM ALFALFA, CLOVER, PEA, AND SOYBEAN

| | |
|--|-----|
| Fig. 1. The Fermentation of Glucose by <i>Rhizobium trifolii</i> in a Yeast Water Medium..... | 251 |
| 2. The Fermentation of Glucose by <i>Rhizobium leguminosarium</i> in a Yeast Water Medium..... | 251 |
| 3. The Fermentation of Glucose by <i>Rhizobium japonicum</i> in a Yeast Water Medium..... | 252 |
| 4. The Fermentation of Glucose by <i>Rhizobium meliloti</i> in a Yeast Water Medium..... | 252 |
| 5. The Fermentation of Xylose by <i>Rhizobium trifolii</i> in a Yeast Water Medium..... | 254 |
| 6. The Fermentation of Xylose by <i>Rhizobium leguminosarium</i> in a Yeast Water Medium..... | 254 |
| 7. The Fermentation of Xylose by <i>Rhizobium japonicum</i> in a Yeast Medium.... | 255 |

| | |
|---|-----|
| 8. The Fermentation of Xylose by <i>Rhizobium meliloti</i> in a Yeast Water Medium..... | 255 |
|---|-----|

DECOMPOSITION OF CITRIC ACID BY SOIL

| | |
|--|-----|
| Fig. 1. Parts per Million—Citric-Acid-Soluble P_2O_5 | 282 |
|--|-----|

CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: V. THE RÔLE OF MICROORGANISMS IN PEAT FORMATION AND DECOMPOSITION

| | |
|--|-----|
| Fig. 1. Course of Decomposition of Sterile Lowmoor Peat by a Soil Suspension and by Pure Cultures of Microorganisms, as Measured by the Liberation of CO_2 | 328 |
| 2. Decomposition of Stems (and Leaves) and Roots (and Rhizones) of <i>Carex</i> (20 Gm. of Dry Matter) and <i>Cladium</i> (2 Gm. of Dry Matter in Sand Cultures) Plants..... | 331 |

COMPOSITION AND NITRIFICATION STUDIES ON *CROTALARIA STRIATA*

| | |
|---|-----|
| Fig. 1. Percentages of Nitrogen Nitrified from the <i>succulent</i> <i>Crotalaria</i> Plant and Its Separate Parts When Incorporated with Norfolk Sandy Soil, as Correlated with the Composition of the Plant and Its Parts..... | 359 |
| 2. Percentages of Nitrogen Nitrified from the Complete <i>Crotalaria</i> Plant and Each of Its Parts When Incorporated with Norfolk Sandy Soil as Correlated with the Composition of the Plant and Each of Its Parts in the <i>early bloom stage</i> of Growth..... | 360 |
| 3. Percentages of Nitrogen Nitrified from the Complete <i>Crotalaria</i> Plant and Each of Its Parts When Incorporated with Norfolk Sandy Soil as Correlated with the Composition of the Plant and Each of Its Parts in the <i>seed pod stage</i> of Growth..... | 361 |
| 4. Percentages of Nitrogen Nitrified from the Complete <i>Crotalaria</i> Plant and Each of Its Parts when Incorporated with Norfolk Sandy Soil as Correlated with the Composition of the Plant and Each of Its Parts in a <i>late fall growth stage</i> or after First Frost..... | 362 |
| 5. Percentages of Nitrogen Nitrified from the Leaves and the Complete Plant When Incorporated with Norfolk Sandy Soil at Different Growth Stages as Correlated with the Composition of the Plant at Such Growth Stages..... | 365 |
| 6. Percentages of Nitrogen Nitrified from the Stems and Roots of the <i>Crotalaria</i> Plant When Incorporated with Norfolk Sandy Soil at Different Growth Stages as Correlated with the Composition of These Parts of the Plant at Such Growth Stages..... | 366 |

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: II. CATAPHORESIS, FLOCCULATION, AND DISPERSION

| | |
|---|-----|
| Fig. 4. The Linear Relation Between the Dissociation of the Colloidal Complex (Values of Z) and the Square Root of the Ionic Strength of the Solution..... | 393 |
| 5. The Relation Between the Size of the Particles, the Surface Density of Ions, and the Force of Cohesion..... | 404 |

NITRATE CHANGES IN A FERTILE SOIL AS INFLUENCED BY SODIUM NITRATE AND AMMONIUM SULFATE

| | |
|---|-----|
| Fig. 1. Nitrate Transformations in 100 gm. Incubated Soils..... | 451 |
|---|-----|

DIURNAL, AVERAGE, AND SEASONAL SOIL TEMPERATURE CHANGES AT DAVIS, CALIFORNIA

| | |
|---|-----|
| Fig. 1. Average Temperatures by 28-Day Periods for 1925..... | 460 |
| 2. Weekly Average Temperatures for the Air, $\frac{1}{2}$ - and 36-Inch Depth Soil Tempera- tures for 1925..... | 461 |
| 3. Average Temperatures by 28-Day Periods for 1927..... | 464 |
| 4. Weekly Average Temperatures for the Air, $\frac{1}{2}$ - and 36-Inch Depths Soil Tempera- tures for 1927..... | 465 |

SOIL PROFILE STUDIES: II. METHODS USED IN THE PROFILE SURVEY OF NEW JERSEY SOILS

| | |
|----------------------------|-----|
| Fig. 1. Profile Types..... | 474 |
|----------------------------|-----|

THE CHEMICAL COMPOSITION OF FLORIDA EVERGLADES PEAT SOILS, WITH SPECIAL REFERENCE TO THEIR INORGANIC CONSTITUENTS

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The Everglades of Florida have, for the past quarter-century, been a topic of much discussion. Large sums of money have been appropriated by the state and national governments for draining and reclaiming this body of organic soils for agricultural purposes. Several drainage canals have been dug, and a branch experiment station has been established by the state for the purpose of studying the problems presented by this vast area.

The Everglades proper occupy the greater portion of south Florida, forming one of the largest bodies of organic soils in the world. They extend from Lake Okeechobee on the north to the Gulf of Mexico on the south, having a length of nearly 100 miles and a maximum width of 50 miles. They have a total area of 7,567 square miles, or 4,843,184 acres of soil material varying from a depth of 8 to 10 feet at Lake Okeechobee to almost nothing at the outer limits. The average depth is about 6 to 8 feet.

Specific information concerning this body of soils is rather limited. The native vegetation and field observations indicate that they are quite different, varying from a state of extremely high natural fertility in the custard apple area, to that of a very low fertility in the saw grass area. The present investigation was undertaken to determine the possible correlation between the natural fertility of these soils and certain chemical properties.

No attempt is made to give a complete review of the literature dealing with the Florida Everglades soils; however, some of the outstanding contributions seem worthy of mention.

Rose has reported a number of chemical analyses of Florida muck and peat soils, including many samples from the Everglades area. The results of his work are given in the Florida state chemist's annual reports for 1912 (10); 1914, 1919, 1920 and 1922 (11). His analyses of muck and peat soils averaged 3.10 per cent ammonia, 0.18 per cent phosphoric acid, and 0.08 per cent potash.

Miller (8), reported a comparison of the chemical properties of saw grass peat with those of the plant from which it was derived, namely saw grass (*Cladium effusum*). He concluded from his results that *seven parts* of saw

¹ The writer wishes to acknowledge the aid and helpful suggestions of Dr. O. C. Bryan of the University of Florida, under whose supervision this work was carried on.

grass were required to produce *one part* of peat, assuming that no silicon was lost in the transformation. On this assumption, 12.2 per cent iron and aluminum, 24 per cent lime, 41 per cent magnesium, 96 per cent potash, 84 per cent soda, 70 per cent phosphorus, and 35 per cent nitrogen were lost during the transformation process.

Waksman (12, 13), reported from a number of decomposition studies on the Florida Everglades soils, that the undrained fibrous peat (saw grass) was high in cellulose (5.6 per cent) whereas the well-drained material (custard apple soil) was free of cellulose.

Forsyth (6), made a biological study of some of the Everglades soils, and concluded that the area known as custard apple soils represented drifted in material to a large extent, whereas the saw grass soils represented a gradual accumulation of plants in place.

Baldwin and Hawker (2), made a survey of a strip of the Florida Everglades soils, beginning on the east side and extending inland to Lake Okeechobee. They described the different soils as peat, peaty muck, and muck, the native vegetation of which is saw grass, elderberry, and custard apple, respectively.

EXPERIMENTAL

Soils used

The soils used in this investigation were classified according to the vegetation found growing on them, as custard apple, elderberry, and saw grass. The greater part of the area of custard apple soil consists of a rim along the southeastern shore of Lake Okeechobee; and the elderberry area forms a belt behind that of the custard apple; whereas the saw grass area lies further from the lake and comprises the greater part of the Everglades proper. Typical soils for each area were selected according to the native vegetation.

The location from which the soils were obtained is given in table 1 and figure 1. Because of the convenience in travel the first four soils of each type were secured near (within 75 to 100 feet) the four main drainage canals; namely, Miami, North New River, Hillsborough, and West Palm Beach. The other samples were secured from representative areas.

Method of collecting samples

The soil samples were secured with an ordinary post-hole digger. The surface vegetation was cleared away and representative samples weighing about two pounds were taken from the first, second, third, fourth, and fifth foot depths, placed in small bags and taken to the laboratory and allowed to air-dry, after which they were ground and stored in quart Mason Jars. Samples were taken from eight typical areas of each of the three types of soil, thus making a total of 120 samples. The surface foot soils were ground to pass an 80-mesh sieve for chemical analyses and specific gravity determinations, but the samples for acidity and ash determinations were not ground.

CHEMICAL COMPOSITION OF THE FLORIDA EVERGLADES SOILS

Standard methods were used for all chemical procedures except that the potash was determined from the ignited soil, and duplicates were made on each determination. The methods used in this study are described by Emerson (5) and Mahin (7).

The insoluble residue was determined by digesting the ash in hydrochloric and nitric acid, after which the residue was washed, ignited, and weighed.

TABLE 1
Location of areas from which samples were obtained

| | | LOCATION |
|----|---------------|--|
| | Custard apple | North New River Canal at South Bay, $\frac{1}{2}$ mile from Lake Okeechobee |
| 2 | Custard apple | Hillsborough Canal at "Chosen" |
| 3 | Custard apple | West Palm Beach Canal at Canal Point |
| 4 | Custard apple | Miami Canal at Miami Locks $\frac{1}{2}$ mile from Lake Okeechobee |
| 5 | Custard apple | North of Hart's Farm, near South Bay |
| 6 | Custard apple | Hart's Farm, between Bell Glade and South Bay. Virgin soil* |
| 7 | Custard apple | Between South Bay and Ritta |
| 8 | Custard apple | Between South Bay and Belle Glade |
| 9 | Elderberry | North New River Canal, $1\frac{1}{2}$ miles south of Lake Okeechobee |
| 10 | Elderberry | Hillsborough Canal, 3 miles from Lake Okeechobee |
| 11 | Elderberry | West Palm Beach Canal at Ertermera Sugar Farm |
| 12 | Elderberry | Miami Canal at Ritta. Virgin soil |
| 13 | Elderberry | Everglades Experiment Station, Grass Garden. Cult. 2 years |
| 14 | Elderberry | Two Miles N. E. Belle Glade, $\frac{1}{2}$ mile into Elderberry area |
| 15 | Elderberry | Hillsborough Canal, 3 miles from Lake Okeechobee |
| 16 | Elderberry | Between Belle Glade and Everglades Experiment Station |
| 17 | Saw grass | North New River Canal at Okeelanta, 4 miles S. Lake Okeechobee |
| 18 | Saw grass | Hillsborough Canal at Everglades Experiment Station |
| 19 | Saw grass | West Palm Beach Canal—Connor's Highway between Canal Point and 20 Mile Bend. 3 miles West of First Gate. |
| 20 | Saw grass | Miami Canal at Ritta. Virgin soil |
| 21 | Saw grass | Geerwarth. Capt. Codd's Place. Cultivated several years |
| 22 | Saw grass | Hillsborough Canal at Six Mile Bridge. |
| 23 | Saw grass | Glade View. Between Belle Glade and 20 Mile Bend |
| 24 | Saw grass | $\frac{1}{2}$ mile North of Everglades Experiment Station |

* All soils had been cultivated some unless otherwise indicated.

The insoluble residue (as SiO_2), N, CaO, MgO , Fe_2O_3 , K_2O , and Al_2O_3 , as found in the different soils, are given in table 2.

The chemical analyses shown in table 2 support the assumption that the custard apple and saw grass soils are two distinct types. This cannot be said, however, of the elderberry soil, which in general appearance, productivity, and location would seem to be a distinct intermediate type.

The chemical analysis of soil 15 (herein classed as elderberry) places it

definitely in the class of the custard apple soils, whereas that of the elderberry soils 11, 12, 13, 14, and 16 is practically identical with the saw grass soils. A truly intermediate type is apparently represented in soils 9 and 10.

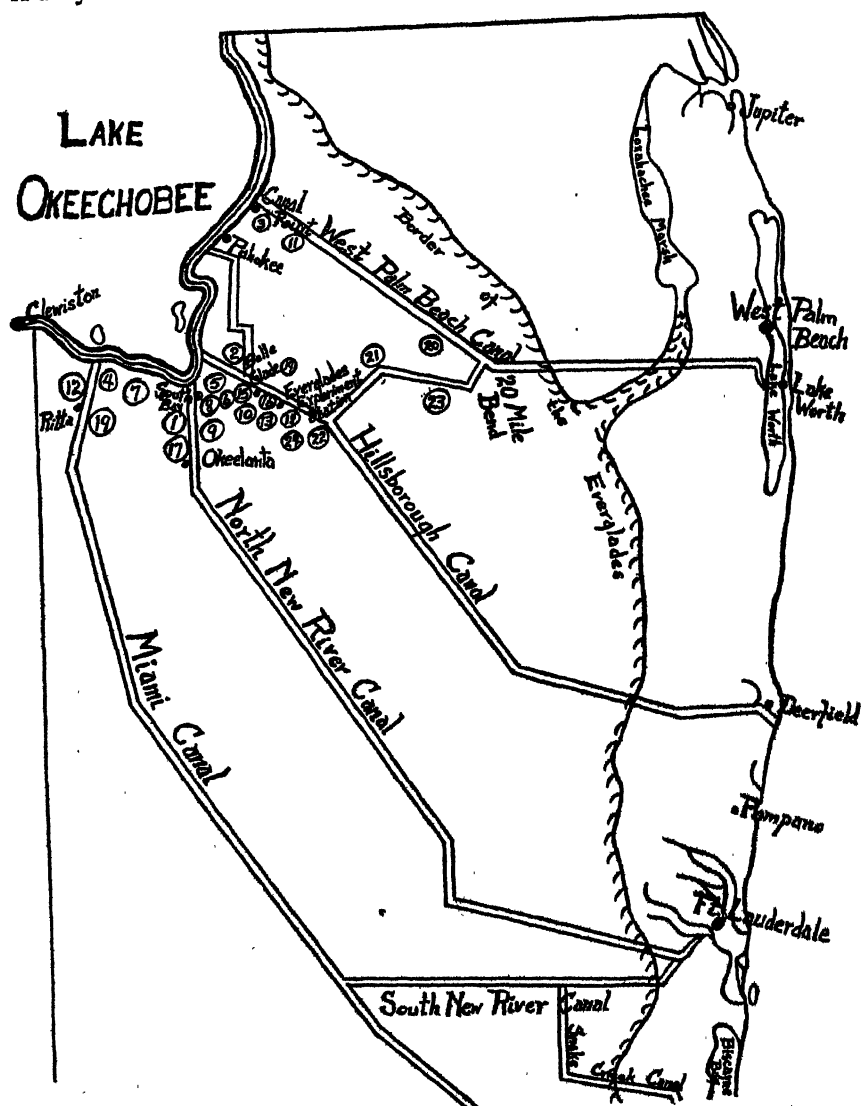


FIG. 1. LOCATION OF SOILS USED IN THIS INVESTIGATION

The custard apple and saw grass soils will be discussed in terms of averages, but obviously averages of the chemical data of the elderberry soils would be meaningless, and will not be considered. The reader can easily see by examin-

ing the various tables that soils 9 and 10 are about half-way between the two extreme types in all physical and chemical comparisons attempted.

It will be noted that the nitrogen in the custard apple soil (1.47 per cent) is very much lower than that in either the elderberry or the saw grass soils (2.79 per cent).

TABLE 2
Chemical analyses of some Everglades soils
(Samples from surface foot of soil, oven-dry basis)

| NUMBER | SAMPLE | SiO ₂ | N | CaO | MgO | Fe ₂ O ₃ | P ₂ O ₅ | K ₂ O | Al ₂ O ₃ |
|-----------------------------|---------------|------------------|-----------------|-----------------|-----------------|--------------------------------|-------------------------------|------------------|--------------------------------|
| | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | Custard apple | 36.02 | 1.315 | 4.500 | 0.0748 | 4.376 | 0.5070 | 0.031 | 4.216 |
| 2 | Custard apple | 40.14 | 1.234 | 4.060 | 0.1110 | 5.354 | 0.4190 | 0.028 | 5.773 |
| 3 | Custard apple | 36.11 | 1.798 | 5.660 | 0.0905 | 3.547 | 0.6160 | 0.052 | 3.128 |
| 4 | Custard apple | 27.06 | 1.891 | 5.140 | 0.0966 | 4.596 | 0.6040 | 0.095 | 3.461 |
| 5 | Custard apple | 25.03 | 1.508 | 3.008 | 0.0845 | 5.427 | 0.4623 | 0.036 | 2.861 |
| 6 | Custard apple | 36.16 | 1.503 | 2.750 | 0.0543 | 5.330 | 0.8022 | 0.050 | 5.068 |
| 7 | Custard apple | 42.11 | 1.411 | 2.529 | 0.1330 | 4.830 | 0.1851 | 0.039 | 5.535 |
| 8 | Custard apple | 43.91 | 1.100 | 2.868 | 0.0604 | 6.826 | 0.2160 | 0.049 | 5.578 |
| Average custard apple. . . | | 35.82 | 1.470 | 3.814 | 0.0881 | 5.036 | 0.4764 | 0.048 | 5.077 |
| 9 | Elderberry | 16.90 | 2.398 | 2.640 | 0.1930 | 2.046 | 0.4610 | 0.087 | 1.297 |
| 10 | Elderberry | 18.99 | 2.406 | 4.860 | 0.0869 | 2.416 | 0.3860 | 0.031 | 2.055 |
| 11 | Elderberry | 1.58 | 2.726 | 6.860 | 0.2530 | 0.539 | 0.4390 | 0.096 | 0.182 |
| 12 | Elderberry | 1.46 | 3.051 | 6.720 | 0.0483 | 0.833 | 0.5940 | 0.041 | 0.000 |
| 13 | Elderberry | 2.28 | 2.612 | 5.317 | 0.1270 | 2.036 | 0.2314 | 0.032 | 0.000 |
| 14 | Elderberry | 5.55 | 2.872 | 5.387 | 0.0664 | 2.201 | 0.3394 | 0.044 | 0.060 |
| 15 | Elderberry | 29.40 | 2.094 | 3.218 | 0.1150 | 2.537 | 0.4163 | 0.054 | 2.597 |
| 16 | Elderberry | 2.86 | 3.001 | 6.996 | 0.1150 | 1.836 | 0.4623 | 0.047 | 0.002 |
| Average elderberry. | | 9.87 | 2.645 | 5.250 | 0.1256 | 1.805 | 0.4165 | 0.054 | 0.777 |
| 17 | Saw grass | 7.14 | 2.683 | 2.930 | 0.1330 | 1.357 | 0.7860 | 0.079 | 0.734 |
| 18 | Saw grass | 3.66 | 2.586 | 10.150 | 0.0814 | 1.171 | 0.5040 | 0.011 | 0.558 |
| 19 | Saw grass | 4.89 | 2.834 | 2.510 | 0.1570 | 1.164 | 0.6860 | 0.043 | 0.000 |
| 20 | Saw grass | 2.24 | 3.020 | 7.320 | 0.0757 | 0.932 | 0.4460 | 0.033 | 0.000 |
| 21 | Saw grass | Lost | 3.231 | 3.988 | 0.1210 | 0.539 | 0.3701 | 0.027 | 0.000 |
| 22 | Saw grass | 1.96 | 2.917 | 5.037 | 0.0604 | 0.140 | 0.2005 | 0.040 | 0.559 |
| 23 | Saw grass | 4.40 | 2.513 | 3.710 | 0.1090 | 1.078 | 0.2005 | 0.034 | 0.142 |
| 24 | Saw grass | 4.97 | 2.569 | 6.017 | 0.0724 | 1.696 | 0.4220 | 0.028 | 0.422 |
| Average saw grass. | | 4.18 | 2.794 | 5.208 | 0.1012 | 1.010 | 0.4156 | 0.037 | 0.302 |

Note: Soils 11, 12, 13, 14, and 16, although placed in the elderberry group because of general appearance, native vegetation, and productivity, are chemically nearly identical with the saw grass soil. Likewise, soil 15 chemically resembles the custard apple muck.

The content of iron, aluminum, and insoluble residue (silica) as noted in table 2, is much higher in the custard apple soil than in the saw grass soils. Calcium and magnesium average a little higher in the saw grass soils than in the custard apple, but there is so much variation that averages mean little.

It is interesting to note that the custard apple soils contain about 16 times as much aluminum and 5 times as much iron as do the saw grass soils. The content of phosphorus does not vary to any marked degree from one type to another. However, there is a considerable variation within the custard apple soil. The content of potash was also variable within the soil type but the average amounts were not very different from type to type.

Reaction

The reaction of the soils was determined according to the Truog method which seemed to give a better estimate of the total acid present than did a measure of the hydrogen-ion concentration. The average pH value of the saw grass soils was 6.7; these soils contain some free calcium carbonate.

The surface soil in practically all instances was more acid than the subsoil. The custard apple soil was strongly acid on the surface, and decreased in acidity with depth, whereas the elderberry and saw grass soils were only slightly acid on the surface and decreased to neutrality at the lower depths.

PHYSICAL PROPERTIES OF THE EVERGLADES SOILS

Fiber content and color

Plate 1 shows the relative amount of undecomposed plant materials (fiber) in the three soils. It will be seen that the original plant material is rather abundant in the saw grass soils, with practically no visible amounts in the custard apple soils. The elderberry soil is intermediate. The custard apple soil was usually black, whereas the other two soils were brown.

Specific gravity

The specific gravity of the surface soils is given in table 4. It will be noted that the specific gravity of the custard apple soils (1.6) is much greater than that of the saw grass soils (1.27).

Structure

The custard apple soils were hard and granular when dry, with very little change upon increase of depth, whereas the elderberry and saw grass soils were comparatively soft and loose. The custard apple soil was also somewhat plastic when wet, in contrast to the other two types.

Water-holding capacity

The hygroscopic and capillary water capacities of the different soils were determined under laboratory conditions, and the results are given in table 4. It will be noted that, in general, the custard apple soils have a somewhat lower content of hygroscopic and much lower content of capillary water than do the other two soils. This is no doubt due to the difference in organic matter present. The saw grass soils had an average of 382.88 per cent capillary and hygroscopic water combined, and the custard apple 223.81 per cent.

Organic matter and ash content

The ash content and loss on ignition of the different soils are given in tables 3 and 4. The custard apple soils averaged 55.48 per cent ash and that of the saw grass soils 12.91 per cent. The loss on ignition was assumed to be practically the same as organic matter. It is worthy of note that the ash content

TABLE 3
Loss on ignition of some Everglades soils
(Percentage oven-dry basis)

| NUMBER | SAMPLE | FIRST FOOT | AVERAGE FIRST FOOT | SECOND FOOT | AVERAGE SECOND FOOT | THIRD FOOT | AVERAGE THIRD FOOT | FOURTH FOOT | AVERAGE FOURTH FOOT | FIFTH FOOT | AVERAGE FIFTH FOOT |
|--------|---------------|------------|--------------------|-------------|---------------------|------------|--------------------|-------------|---------------------|------------|--------------------|
| 1 | Custard apple | 42.59 | | 45.88 | | 57.19 | | 72.23 | | 87.60 | |
| 2 | Custard apple | 34.88 | | 35.92 | | 36.38 | | 28.81 | | 20.00 | |
| 3 | Custard apple | 48.26 | | 44.09 | | 34.77 | | 29.54 | | 32.63 | |
| 4 | Custard apple | 56.69 | 44.52 | 41.40 | 44.34 | 42.66 | 44.84 | 46.60 | 46.34 | | 47.90 |
| 5 | Custard apple | 47.14 | | 50.42 | | 51.59 | | 31.30 | | 32.23 | |
| 6 | Custard apple | 46.36 | | 41.80 | | 53.98 | | 61.69 | | 33.59 | |
| 7 | Custard apple | 43.57 | | 52.42 | | 40.31 | | 54.62 | | 82.58 | |
| 8 | Custard apple | 36.70 | | 42.02 | | 41.80 | | 45.92 | | 46.69 | |
| 9 | Elderberry | 70.58 | | 69.74 | | 68.80 | | 75.69 | | 86.35 | |
| 10 | Elderberry | 68.08 | | 51.10 | | 57.19 | | | | | |
| 11 | Elderberry | 89.52 | | 90.54 | | 85.91 | | 72.06 | | 65.68 | |
| 12 | Elderberry | 89.94 | 80.15 | 91.09 | 74.84 | 90.59 | 77.65 | 90.15 | 83.43 | 91.27 | 81.36 |
| 13 | Elderberry | 88.96 | | 88.97 | | 88.55 | | 87.77 | | 88.36 | |
| 14 | Elderberry | 84.11 | | 80.10 | | 90.68 | | 84.78 | | 68.29 | |
| 15 | Elderberry | 61.63 | | 45.07 | | 51.78 | | | | | |
| 16 | Elderberry | 88.34 | | 82.06 | | 87.70 | | 90.10 | | 88.25 | |
| 17 | Saw grass | 81.38 | | 90.53 | | 91.00 | | 63.19 | | 86.44 | |
| 18 | Saw grass | 82.33 | | 65.63 | | 57.08 | | 68.99 | | 68.05 | |
| 19 | Saw grass | 86.08 | | 91.81 | | 90.62 | | 92.69 | | 93.73 | |
| 20 | Saw grass | 87.93 | 87.09 | 90.51 | 86.58 | 90.72 | 85.23 | 91.01 | 83.30 | 93.33 | 86.44 |
| 21 | Saw grass | 90.51 | | 93.15 | | | | | | | |
| 22 | Saw grass | 89.39 | | 81.70 | | 87.40 | | 87.41 | | 89.63 | |
| 23 | Saw grass | 90.82 | | 91.67 | | 93.10 | | 95.12 | | 94.98 | |
| 24 | Saw grass | 88.25 | | 87.68 | | 86.71 | | 84.70 | | 78.94 | |

at different depths in the same soil type was quite variable and irregular. Seven soils showed a decrease of ash with depth whereas five others showed an increase with depth. Of the remaining twelve soils, ten were irregular in their variation and two (with about 10 per cent ash) had about the same percentage at all depths.

DISCUSSION

From the chemical and physical characteristics of the custard apple soils, it would seem that these soils were formed either from the drifting in of ex-

TABLE 4
Specific gravity, hygroscopic and capillary water, loss on ignition, and ash content of some Everglades soils

(Samples from surface foot, per cent oven-dry basis)

| NUMBER | SAMPLE | SPECIFIC GRAVITY | HYGROSCOPIC AND CAPILLARY WATER | | LOSS ON IGNITION | ASH CONTENT |
|----------------------------|---------------|------------------|---------------------------------|-----------------|------------------|-----------------|
| | | | Hygroscopic | Capillary | | |
| | | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | Custard apple | 1.62 | 10.51 | 207.96 | 42.59 | 57.41 |
| 2 | Custard apple | 1.60 | 12.32 | | 34.88 | 65.12 |
| 3 | Custard apple | 1.49 | 10.84 | 241.90 | 48.26 | 51.74 |
| 4 | Custard apple | 1.50 | 11.17 | 227.72 | 56.69 | 43.31 |
| 5 | Custard apple | 1.62 | 13.83 | | 47.14 | 52.86 |
| 6 | Custard apple | 1.63 | 14.06 | 216.32* | 46.36 | 53.64 |
| 7 | Custard apple | 1.65 | 13.06 | | 43.57 | 56.43 |
| 8 | Custard apple | 1.73 | 12.40 | 225.18 | 36.70 | 63.30 |
| Average custard apple..... | | 1.60 | 12.25 | 223.81 | 44.52 | 55.48 |
| 9 | Elderberry | 1.35 | 18.03 | 326.33 | 70.58 | 29.42 |
| 10 | Elderberry | 1.32 | 14.47 | 366.65 | 68.08† | 31.92 |
| 11 | Elderberry | 1.22 | 18.28 | | 89.52 | 10.48 |
| 12 | Elderberry | 1.22 | 14.20 | 289.19* | 89.94 | 10.06 |
| 13 | Elderberry | 1.27 | 16.24 | | 88.96 | 11.04 |
| 14 | Elderberry | 1.17 | 15.75 | 380.14 | 84.11 | 15.89 |
| 15 | Elderberry | 1.46 | 14.44 | 340.34* | 61.63 | 38.37 |
| 16 | Elderberry | 1.31 | 15.52 | | 88.34 | 11.66 |
| Average elderberry..... | | 1.29 | 15.85 | 336.53 | 80.15 | 19.85 |
| 17 | Saw grass | 1.23 | 16.50 | 410.13 | 81.38 | 18.62 |
| 18 | Saw grass | 1.27 | 18.10 | | 82.33 | 17.67 |
| 19 | Saw grass | 1.25 | 14.33 | 370.86 | 86.08 | 13.92 |
| 20 | Saw grass | 1.20 | 22.36 | 373.56* | 87.93 | 12.07 |
| 21 | Saw grass | 1.28 | 16.16 | | 90.51 | 9.49 |
| 22 | Saw grass | 1.28 | 16.26 | 295.76 | 89.39 | 10.61 |
| 23 | Saw grass | 1.29 | 12.05 | 364.10 | 90.82 | 9.18 |
| 24 | Saw grass | 1.34 | 16.00 | | 88.25 | 11.75 |
| Average saw grass..... | | 1.27 | 16.47 | 382.88 | 87.09 | 12.91 |

* Soils nos. X, 26, IX, and XI respectively in order listed—not included in table 1.

† See note under table 2.

traneous materials from the flooded waters of Lake Okeechobee, or represent an advanced stage of decomposition of materials which formed the saw grass soils.

The theory that the custard apple soil represents an advanced stage of decomposition would gain some support from the fact that the percentages of silicon, iron, and aluminum are much higher in these soils, and from the presence of very large amounts of fibrous material in the saw grass soils compared with little or none in the custard apple type. Such a theory, however, gains little support from the fact that there is little difference in the ratio between the organic matter and nitrogen in the two types of soils. The two soils do not differ greatly in the average amounts of phosphorus, potassium, magnesium, and calcium.

The presence of the larger amounts of silicon, aluminum, and iron in the custard apple soils could be explained by assuming that these elements were washed in by over-flow water from Lake Okeechobee. This agrees well with the fact that the custard apple soil is found in a narrow strip parallel to and adjoining the lake shore and is found to be much wider on the southeastern side where the low sand ridge, which practically surrounds the lake, is absent. Forsaith's (6) morphological studies show that the sedimentary soils of which the custard apple soil is a type, contains large amounts of diatoms, which were probably carried in by water.

The saw grass soil was found by Waksman (13) to contain considerable amounts of cellulose (5.22 per cent) whereas the custard apple type was found to be devoid of cellulose. This cannot be attributed to a greater decomposition change in the custard apple soils, since it has not been sufficient to change greatly the ratio of organic matter to nitrogen.

The high content of silicon, aluminum, and iron in the custard apple soils together with the non-fibrous organic matter, having a nitrogen content similar to that of the organic matter of the saw grass soils, indicates strongly that the former are in the main sedimentary in nature.

Although as a whole the custard apple soils appear to be in a slightly advanced stage of decomposition, an examination of the soil profile shows that as a rule there is a brown and fibrous strata, containing a low ash content, within the 5-foot depths. On the other hand a dark plastic layer is often found in the lower depths of the saw grass soil. This zonification would indicate that different strata or layers were formed under different conditions of drainage, and from a different type of material at that period of formation.

Miller (8) and Baldwin and Hawker (2), report an increase in ash content upon increase in depth. It has been previously indicated that the soils herein reported are very irregular in this respect. However, their samples were taken at much greater depth (10 and 11 feet) than those used in this investigation.

The reaction of the soils, seems to indicate that there has been some leaching of carbonates, especially in the custard apple soils. The decrease in acidity upon increase in depth was doubtless due to the presence of calcium carbonate at the lower levels. The calcium content in the lower depth was not determined, but Miller (8) reports more calcium in the subsoil of saw grass peat than in the surface, and attributes it to leaching. It may be that an upward

movement of calcium from the rock beneath accounts for the apparently high content and lower acidity at the lower zones.

The specific gravity and degree of hardness were in proportion to the ash content. The water-holding capacity is not in accord with the results of Wheeler (14), who reports that the water-holding capacity of a peat is 600 per cent. Beattie (3) reports that air-dry peat will absorb eight times its weight in water. These results were probably obtained from different types of material, which would no doubt affect the absorptive power of the soil. Whitson and Walster (15) claim that the water-holding capacity of a peat varies from 201 to 309 per cent. Their results agree very closely with those in this study. The water-holding capacity of the different soils is roughly in proportion to the amount of organic matter present.

The results do not indicate that the poor plant growth on the saw grass soils is due to a deficiency of the usual fertilizer constituents, nitrogen, phosphorus, and potassium. These soils are made productive, however, by the use of small amounts of copper and manganese sulfates (1). The function of these chemicals in this case is not known at present.

SUMMARY

Twenty-four representative soils from the Florida Everglades were selected for this study. They were classified according to the native vegetation growing on them; namely, saw grass, elderberry, and custard apple. Eight representative areas to a depth of five feet each, of these three types of soil, were used.

The chemical analyses and specific gravity determinations were made from the surface foot soils only, while the ash and acidity determinations were made on all the soils at the various depths. The results are as follows:

1. The custard apple and saw grass soils were found to represent two chemically distinct types. The results place the former in the class of true *mucks*, and the latter, true *peats*. The results indicate that the custard apple soils are sedimentary in nature, and the saw grass accumulative.

2. The elderberry soil is not so distinct, from this standpoint. Soils 11, 12, 13, 14, and 16, are practically identical in analysis with the saw grass soils, whereas soil 15 would be placed with the custard apple group. Two soils, 9 and 10, are chemically intermediate between the two extreme types.

3. The custard apple and saw grass soils averaged 35.81 per cent, and 4.12 per cent silicon dioxide; 5.077 per cent, and 0.301 per cent aluminum oxide; and 5.036 per cent and 1.010 per cent iron oxide.

4. The custard apple and saw grass soils averaged 1.470 per cent, and 2.784 per cent nitrogen; 0.4764 per cent and 0.4156 per cent phosphorus pentoxide; and 0.048 per cent and 0.037 per cent potassium oxide, in order named.

5. The average calcium oxide content for the custard apple and saw grass soils was 3.814 per cent, and 5.208 per cent respectively, whereas the magnesium oxide content was 0.0881 per cent and 0.0102 per cent respectively.

The reaction of the custard apple soil was acid, whereas elderberry and saw grass soils were

slightly acid to neutral. The acidity decreased upon increase in depth, whereas the calcium and magnesium probably increased.

6. The custard apple and saw grass soils contained 44.52 per cent and 86.37 per cent organic matter; 12.25 per cent and 16.47 per cent hygroscopic water; and 223.81 per cent and 382.88 per cent capillary water respectively.

7. The saw grass and elderberry soils are light brown to dark brown in color, light, loose, and soft; whereas those of the custard apple are black, heavy, granular, and hard.

8. The irregularity of color, fiber, and ash content of the profiles of these soils indicate alternate sedimentation and cumulation of layers or zones in the soil.

9. The custard apple soils have a higher natural fertility than the saw grass soils, but the latter are rendered productive by the use of copper and manganese salts. The poor fertility of the saw grass soil is not due to the absence of the usual fertilizer constituents, nitrogen, phosphorus, and potassium.

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PLATE 1

RELATIVE AMOUNTS OF PARENT PLANT MATERIAL IN EVERGLADES SOILS

(The custard apple soil contains very little fibrous material, whereas the elderberry and saw grass soils contain relatively high amounts.)



No. 1. Saw Grass



No. 2. Elderberry



No. 3. Custard Apple

VARIATIONS IN THE CALCIUM AND MAGNESIUM CONTENTS OF PEA PLANTS ON DIFFERENT SOIL TYPES¹

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Two previous papers (2, 3) contained data showing variations in the calcium and magnesium contents of alfalfa and bean plants when grown on different soil types, and also showing the relationship of these to some properties of the soils. The material presented here is of the same nature in regard to the pea plant and completes the material dealing with the effect of soil type upon certain of the characteristics of three important legumes.

Because of their wide range of adaptability, field peas constitute one of the leading sources of legume forage, and likewise one of the important leguminous crops used widely in farm rotations looking to the maintenance of soil fertility. Because of these facts any information related to variations in the composition of field peas, and causes inducing them, has a practical significance.

A rather comprehensive review of the work which has been done relative to the discussion taken up here was given in the aforesaid papers on alfalfa and beans, obviating any need for such review in this paper. Likewise the plan of the experiment was the same as that followed for beans (3) and the experimental methods were the same as reported in the other two papers. These exceptions should be noted however, that Roselawn soil was not included in the work with peas, reducing the number of soils to six, and also that the period of growth of the peas was prolonged so greatly, probably because of abnormal photoperiodism, that three stages of growth were obtained before budding and no samples were taken at the stage when the fruit was setting on. The variety of peas used was Scotch green field peas.

As in the case of the work reported on beans, a knowledge of the effect of plant growth on the soil characteristics studied here is necessary to an understanding of the other material. For this reason a discussion of these data will be given first.

EFFECT OF GROWTH OF PEAS UPON CALCIUM AND MAGNESIUM CONTENTS OF SOIL SOLUTIONS AND UPON pH VALUES OF SOILS

Very much the same effect upon the calcium and magnesium contents of the soil solution was produced by the growing pea plants, shown in tables 1 and 2,

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as was found in the case of growing bean plants (3). Although it is evident that before growth started the amounts of calcium and magnesium present in the several soil solutions varied greatly, by the time the pea plants had reached the mature stage the amounts had been reduced until there was but little difference among the soil types. However, the reduction in the concentration of the two elements in the solutions was not rapid and was very uniform, the soil types maintaining the same order with respect to each other during most

TABLE 1
Variations in calcium content of soil solutions growing peas
Parts per million of water-free soil

| SOIL TYPE | INITIAL CONTENT | STAGES OF GROWTH | | |
|-----------------|-----------------|--------------------|--------------------|---------------------|
| | | Content at 3 weeks | Content at 6 weeks | Content at maturity |
| Plainfield..... | 10.60 | 2.99 | 4.85 | 3.02 |
| Kewanee..... | 44.70 | 38.80 | 21.00 | 1.60 |
| Onaway..... | 24.55 | 20.95 | | 1.93 |
| Hillsdale..... | 14.62 | 12.81 | 7.00 | 1.92 |
| Brookston..... | 19.10 | 8.22 | 10.12 | 5.10 |
| Miami..... | 30.06 | 23.35 | 18.50 | 3.13 |

TABLE 2
Variations in magnesium content of soil solutions growing peas
Parts per million of water-free soil

| SOIL TYPE | INITIAL CONTENT | STAGES OF GROWTH | | | |
|-----------------|-----------------|--------------------|--------------------|--------------------|---------------------|
| | | Content at 3 weeks | Content at 6 weeks | Content at 8 weeks | Content at maturity |
| Plainfield..... | 4.44 | 3.18 | Trace | 0.66 | 1.90 |
| Kewanee..... | 8.88 | 4.55 | 4.99 | 1.87 | Trace |
| Onaway..... | 7.45 | 5.11 | | 1.33 | 1.55 |
| Hillsdale..... | 4.08 | 2.39 | 0.98 | 2.52 | 1.71 |
| Brookston..... | 3.48 | 0.92 | Trace | Trace | Trace |
| Miami..... | 10.2 | 5.81 | 1.11 | 2.05 | 1.61 |

of the growth period. It appears that pea plants reduced the calcium content of the soil solution less rapidly, but to a greater degree, than had been found for bean plants, although the effect of the two crops on the magnesium content of the soil solutions was found to be about the same. It thus appears from tables 1 and 2 that in point of concentration of calcium and magnesium in their solution the soils may be considered as decreasing in the order of Kewanee, Onaway, Hillsdale, and Plainfield for the light soils, and of Miami and Brookston for the heavy soils.

CHANGES INDUCED IN THE HYDROGEN-ION CONCENTRATION OF THE SOILS BY THE GROWTH OF PEA PLANTS

The hydrogen-ion concentration of the soils, represented by the pH values in table 3, appears to have fluctuated considerably during the growth period of the plants. Rather larger variations appeared than can be attributed to other contributory causes, and it is evident that the pH values of the strongly acid soils were raised whereas those of the alkaline or neutral soils were decreased by the growing plants. This was not found true for beans (3) but appears to be in order with the work reported by Arrhenius (1). However, throughout the growth period the soils maintained their relative positions in respect to their hydrogen-ion concentrations and can be considered as in the order given in table 3, where they are grouped into light and heavy soils.

TABLE 3
Effect of growth of peas on the pH value of the soil

| SOIL TYPE | INITIAL VALUE | STAGES OF GROWTH | | | | |
|--------------------|------------------|------------------------|------------------------|------------------------|--------------------------|---------------------------|
| | | Value at 3 weeks | Value at 6 weeks | Value at 8 weeks | Value at bud- ding | Value at ma- turity |
| | pH | pH | pH | pH | pH | pH |
| Onaway..... | 7.40 | 7.15 | | 7.50 | 7.14 | 7.17 |
| Plainfield..... | 7.35 | 7.69 | 7.40 | 7.37 | 6.99 | 6.95 |
| Hillsdale..... | 6.25 | 6.12 | 6.18 | 6.64 | 6.50 | 5.96 |
| Kewanee..... | 5.10 | 5.11 | 5.00 | 5.80 | | 5.85 |
| <i>Heavy soils</i> | | | | | | |
| Brookston..... | 7.05 | 7.33 | 7.01 | 7.15 | | 6.83 |
| Miami..... | 5.00 | 5.18 | 4.80 | 5.05 | | 5.38 |

CALCIUM CONTENT OF GREEN PEA STEMS AND LEAVES AT DIFFERENT STAGES OF GROWTH WHEN GROWN ON DIFFERENT SOIL TYPES

Stems

Noticeable differences were found in the calcium content of the stems of green pea plants grown on the different soil types, as is shown in table 4. Generally the stems of plants grown on any one soil were quite uniformly either high or low in calcium in relation to the stems of plants grown on the other soils. But it is readily apparent that the calcium content of the stems did not depend entirely upon the concentration of this element in the soil solution, nor upon either the pH value of the soils or their textures. It does appear however, that all three of these factors may have played a part in controlling the amounts of calcium contained in the stems. Thus, the calcium content was generally high in the stems of plants grown on the heaviest soil types, but always one of the lighter soils produced plants the stems of which were likewise

high in calcium. Also, if only the light textured soils are considered, and if Onaway and Plainfield are recognized as alkaline, and Kewanee and Hillsdale as acid, it becomes apparent that the soils of similar hydrogen-ion concentrations produced pea plants whose stems usually contained calcium in proportion to the amounts present in the soil solutions. This relationship cannot be applied to the Miami and Brookston soils studied.

Considerable variation occurred in the calcium content of the pea stems at different stages of growth, but there was no uniform increase or decrease as the growth period advanced. In the stems of plants grown on four of the soil types, less calcium was present in the green material at maturity than at the beginning of growth, but in the other two samples there was more present.

TABLE 4
Calcium content of green pea stems at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|------------------------------|-----------------|----------|----------|----------|----------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | per cent | per cent | per cent | per cent | per cent |
| <i>Light, alkaline soils</i> | | | | | |
| Onaway..... | 0.181 | | 0.124 | 0.140 | 0.208 |
| Plainfield..... | 0.167 | 0.139 | 0.157 | 0.110 | 0.121 |
| <i>Light, acid soils</i> | | | | | |
| Kewanee..... | 0.135 | 0.173 | 0.146 | | 0.191 |
| Hillsdale..... | 0.144 | 0.135 | 0.087 | 0.102 | 0.132 |
| <i>Heavy soils</i> | | | | | |
| Miami..... | 0.162 | 0.233 | 0.168 | | 0.108 |
| Brookston..... | 0.174 | 0.176 | 0.150 | | 0.128 |

Leaves

It is apparent from the data in table 5 that similar variations occurred in the calcium content of the green material of pea leaves as was found in that of the green material of pea stems. Differences of considerable magnitude appeared in the calcium content of the leaves obtained from the different soil types throughout the period of growth, and these became greater toward the close of the growth period.

Although it is evident that the calcium present in the pea leaves did not correspond entirely to the concentration of this element in the soil solutions, when the soils were of similar texture and hydrogen-ion concentration, the amount of calcium contained in the green leaves was higher when the amount contained in the soil solution was high, and vice versa. It is noticeable that

the average percentage of calcium in the leaves grown on alkaline soils was greater than that of the leaves grown on the acid soils, where Brookston and Miami soils are not considered. In these cases the situation was reversed.

Much greater amounts of calcium were present in the mature green pea leaves than in the young leaves and, although not entirely uniform throughout the period of growth, the increase of calcium was generally quite rapid.

Larger amounts of calcium were present in the leaves of peas than in the stems during the entire period of growth and the ratio between the amounts in the two plant parts became much wider toward maturity.

TABLE 5

Calcium content of green pea leaves at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| <i>Light, alkaline soils</i> | | | | | |
| Onaway..... | 0.259 | | 0.260 | 0.462 | 0.536 |
| Plainfield..... | 0.193 | 0.286 | 0.285 | 0.379 | 0.384 |
| <i>Light, acid soils</i> | | | | | |
| Kewanee..... | 0.196 | 0.359 | 0.274 | 0.292 | 0.418 |
| Hillsdale..... | 0.185 | 0.332 | 0.254 | 0.292 | 0.282 |
| <i>Heavy soils</i> | | | | | |
| Miami..... | 0.197 | 0.384 | 0.278 | | 0.582 |
| Brookston..... | 0.193 | 0.293 | 0.336 | | 0.436 |

CALCIUM CONTENT OF THE EXPRESSED JUICE OF PEA LEAVES AND STEMS AT
DIFFERENT STAGES OF GROWTH ON THE DIFFERENT SOIL TYPES

Juice of stems

Significant differences were found in the amounts of calcium in the expressed juice of pea stems of plants obtained from the different soil types, as is shown in table 6. These data are marked by considerable lack of uniformity among the different soil types, no one type giving stems with juice consistently higher or lower in calcium than any of the other soil types.

The same relationship between the calcium content of the juice and the properties of the soils studied is evident here as was noted in the case of the calcium content of the green tissue of stems, except that variations in texture had less influence than in the other case. But on soils of similar reaction the calcium content of the expressed juice usually varied directly as the concentra-

tion of calcium in the soil solution. Where soil texture and more particularly soil reaction, are disregarded there appears to have been no relationship between the amounts of calcium in the soil solutions and those in the juice of the plants.

In plants grown on four of the soil types the concentration of calcium was less in the juice of mature stems than in that of young stems, whereas in the plants from the other two types it was slightly higher. During the intermediate growth period the concentration of calcium varied greatly, but appeared to reach its highest point when the plants were 6 to 8 weeks old.

Juice of leaves

Although marked differences were found in the amounts of calcium present in the juice of pea leaves obtained from plants grown on the different soil

TABLE 6
Calcium content of the expressed juice of pea stems at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| <i>Alkaline soils</i> | | | | | |
| Onaway..... | 0.164 | | 0.114 | 0.109 | 0.142 |
| Brookston..... | 0.125 | 0.143 | 0.114 | | 0.093 |
| Plainfield..... | 0.125 | 0.132 | 0.136 | 0.071 | 0.104 |
| <i>Acid soils</i> | | | | | |
| Kewanee..... | 0.128 | 0.173 | 0.105 | | 0.220 |
| Miami..... | 0.121 | 0.185 | 0.145 | | 0.142 |
| Hillsdale..... | 0.103 | 0.177 | 0.065 | 0.086 | 0.095 |

types, as appears in table 7, there was so much fluctuation during the growth period that it appears to be impossible to associate the amounts present with any of the soil properties which were considered. Likewise it is only possible to say that in the plants obtained from all of the soil types the concentration of calcium was greater in the juice expressed from the mature leaves than in that expressed from the young leaves and that it varied greatly because of soil type during the intermediate period of growth.

The concentration of calcium was greater in the expressed juice of the leaves at all times than in that of the stems and the difference became greater as the growth period advanced.

MAGNESIUM CONTENT OF GREEN PEA STEMS AND LEAVES AT DIFFERENT STAGES OF GROWTH ON DIFFERENT SOIL TYPES

The magnesium content of plants studied previously (2, 3) was found to fluctuate so much that any study of its relationship to modifying factors was difficult. A cursory examination of the data presented in the following pages indicates that such difficulty is to be encountered here.

Stems

In table 8 are given the data representing the magnesium contents of the stems of green pea plants at different stages of growth on the several soil types.

TABLE 7
Calcium content of the expressed juice of pea leaves at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| <i>Alkaline soils</i> | | | | | |
| Onaway..... | 0.206 | | | 0.372 | 0.126 |
| Brookston..... | 0.145 | 0.260 | 0.235 | | 0.358 |
| Plainfield..... | 0.206 | 0.236 | 0.170 | 0.260 | 0.272 |
| <i>Acid soils</i> | | | | | |
| Kewanee..... | 0.162 | 0.298 | 0.184 | | 0.382 |
| Miami..... | 0.147 | 0.315 | 0.185 | | 0.558 |
| Hillsdale..... | 0.098 | 0.296 | 0.223 | 0.205 | 0.199 |

Great differences existed in the amounts of magnesium present in the plant material obtained from the different soils and the greatest range of variation appeared during the intermediate stages of growth, the contents at the beginning and close of the growth period being more uniform.

Generally a larger percentage of magnesium was present in the stems obtained from the heavy soils during the early part of the growth period. There appears to have been a tendency for the amount of magnesium to increase in the stems of plants grown on the sandy soils as the growth period advanced, whereas a decrease occurred in the stems of plants obtained from the heavy soils. This resulted in about a uniform content at maturity as far as the effect of soil texture was concerned.

There is no evidence that the magnesium content of the pea stems was influenced by either the reaction of the soil or the concentration of magnesium in the soil solution. Likewise the data given here and those in table 1 indicate that there was no relationship between the amounts of magnesium and the amounts of calcium present in the pea stems.

Leaves

Apparently the magnesium content of the leaves of pea plants grown on the different soil types was more uniform than that of the stems of the same plants. This is brought out by the data in table 9, where differences of less magnitude are evident throughout the period of growth.

The amounts of magnesium present in the green pea leaves varied independently of any of the soil characteristics studied here, insofar as could be determined by the data of table 9.

TABLE 8

Magnesium content of green pea stems at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------|-----------------|----------|----------|----------|----------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | per cent | per cent | per cent | per cent | per cent |
| Plainfield..... | 0.039 | 0.017 | 0.043 | 0.046 | 0.052 |
| Hillsdale..... | 0.052 | 0.013 | 0.017 | 0.046 | 0.039 |
| Kewanee..... | 0.032 | 0.066 | 0.026 | | 0.053 |
| Onaway..... | 0.062 | | 0.037 | 0.052 | |
| Brookston..... | 0.066 | 0.077 | 0.043 | | 0.052 |
| Miami..... | 0.066 | 0.046 | 0.026 | | 0.039 |

TABLE 9

Magnesium content of green pea leaves at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------|-----------------|----------|----------|----------|----------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | per cent | per cent | per cent | per cent | per cent |
| Plainfield..... | 0.055 | 0.108 | 0.061 | 0.105 | 0.096 |
| Hillsdale..... | 0.063 | 0.099 | 0.052 | 0.079 | 0.083 |
| Kewanee..... | | 0.066 | 0.057 | | 0.070 |
| Onaway..... | 0.076 | | 0.053 | 0.108 | 0.109 |
| Brookston..... | 0.063 | 0.079 | 0.087 | | 0.118 |
| Miami..... | 0.071 | 0.082 | 0.052 | | 0.257 |

Greater amounts of magnesium were present in the green material of pea leaves than in that of pea stems at each stage of growth. Likewise there was an increased amount present in mature leaves as compared with that of young leaves on all of the soil types studied, resulting in a wider ratio in the two plant parts at maturity than in the early stages of growth.

MAGNESIUM CONTENT OF THE EXPRESSED JUICE OF PEA STEMS AND LEAVES

Stems

Aside from the fact that the magnesium content of the juice of pea stems varied greatly in the plants grown on the different soil types, there is only one

significant feature brought out by the data of table 10. It is strikingly evident that the magnesium content of the juice of the stems obtained from plants grown on the two very acid soils was very low when the plants were 3 weeks old. However, on these soils a greater increase in concentration occurred in the magnesium content of the juice, resulting in a greater percentage of magnesium at maturity in the samples obtained from the very acid soils. Just what property of the soils or the plants induced this condition is not indicated.

TABLE 10
Magnesium content of the expressed juice of pea stems at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------|-----------------|----------|----------|----------|----------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | per cent | per cent | per cent | per cent | per cent |
| Plainfield..... | 0.058 | 0.037 | 0.037 | 0.028 | 0.043 |
| Hillsdale..... | 0.045 | 0.053 | 0.025 | 0.028 | 0.032 |
| Kewanee..... | 0.018 | 0.039 | 0.021 | | 0.064 |
| Onaway..... | 0.057 | | 0.027 | 0.036 | 0.048 |
| Brookston..... | 0.056 | 0.066 | 0.031 | | 0.041 |
| Miami..... | 0.011 | 0.067 | 0.043 | | 0.058 |

TABLE 11
Magnesium content of expressed juice of pea leaves at different stages of growth, on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | |
|-----------------|-----------------|----------|----------|----------|----------|
| | 3 weeks | 6 weeks | 8 weeks | Budding | Maturity |
| | per cent | per cent | per cent | per cent | per cent |
| Plainfield..... | 0.049 | 0.071 | 0.056 | 0.053 | 0.059 |
| Hillsdale..... | 0.023 | 0.070 | 0.067 | 0.062 | 0.058 |
| Kewanee..... | 0.021 | 0.056 | 0.036 | | 0.059 |
| Onaway..... | 0.037 | | 0.062 | 0.088 | 0.039 |
| Brookston..... | 0.059 | 0.076 | 0.069 | | 0.092 |
| Miami..... | 0.059 | 0.078 | 0.051 | | 0.171 |

Evidently there was no relationship between the texture of the soil or the amounts of magnesium present in the soil solutions and the concentration of magnesium in the juice of the stems.

Leaves

In table 11 are given the data showing the magnesium content of the expressed juice of the pea leaves. Marked differences existed in the magnesium contents of the juice of the leaves obtained from the different soil types and it appears that these contents were greater in the plants grown upon the heavy soil types during the entire growth period than they were in the plants obtained

from the light soils. However, there was no correlation between the magnesium present in the plant juice and that present in the soil solution, nor was there evident any influence of the reaction of the soil upon the amount of magnesium in the plant juice.

In the early stages of growth the concentration of magnesium was about equal in the juice of the stems and leaves but as the growth period advanced the concentration increased more rapidly in the juice of the leaves with the result that in the mature stage it was higher here.

All of the data obtained in this work showed that there was always more calcium than magnesium present in the green plant material and in the juice of the pea plant.

TABLE 12
Green weight of peas at different stages of growth on different soil types
Weight in grams of 7 plants

| SOIL TYPE | STAGE OF GROWTH | | |
|-----------------|-----------------|---------|---------|
| | 3 weeks | 6 weeks | 8 weeks |
| | gm. | gm. | gm. |
| Plainfield..... | 9.10 | 21.7 | 65.5 |
| Kewanee..... | 7.28 | 16.9 | 61.2 |
| Onaway..... | 7.25 | | 54.6 |
| Hillsdale..... | 11.90 | 29.0 | 82.7 |
| Brookston..... | 8.26 | 21.0 | 46.5 |
| Miami..... | 7.14 | 9.1 | 27.7 |

GREEN WEIGHT OF PEAS ON DIFFERENT STAGES OF GROWTH ON DIFFERENT
SOIL TYPES

The green weights of pea plants produced on the different soil types were obtained for only three stages of growth, because of the extent to which the leaves dropped as the plants approached maturity. These data appear in table 12.

When the plants were 3 weeks old, their green weight was nearly equal on all of the soil types, but as the growth period advanced, the rate of growth changed markedly. Plants grown on Hillsdale and Plainfield soils made the most rapid growth and those grown on Miami and Brookston soils made the least rapid growth. The data showing the calcium and magnesium contents of the plants indicate that the rapidly growing plants were usually low in these two elements, whereas the slowly growing plants were usually high in them. It thus appears that at least some of the differences noted in the calcium and magnesium contents of the plants may be attributed to differences in the rate at which the plants were making growth as well as to the differences in the characteristics of the soils.

PROPORTION OF LEAVES AND STEMS OF PEAS GROWN IN THE GREENHOUSE ON
DIFFERENT SOIL TYPES

In table 13 are given the green weights of the stems and leaves of peas at several growth stages. At 3 weeks of age there was little variation in the proportion of leaves and stems on the different soil types, but as the plants became older, greater differences appeared.

There was an increase in the proportion of stems to leaves during the period of growth until it became about constant at the budding stages. In the beginning the average percentages were 40 for stems and 60 for leaves. When the plants were 8 weeks old, the average percentage of stems had become 58.2 and that of leaves 41.8; which about reversed the situation found at the age of 3 weeks. At the 3-week stage of growth the proportion of stems and leaves in peas was about the same as was found for beans, but in beans the proportion of stems decreased, while in peas it increased with the age of the plant.

TABLE 13

Proportion of leaves and stems of peas at different stages of growth on different soil types

| SOIL TYPE | STAGE OF GROWTH | | | | | | | |
|-----------------|-----------------|----------|----------|----------|----------|----------|----------|----------|
| | 3 weeks | | 6 weeks | | 8 weeks | | Budding | |
| | Stems | Leaves | Stems | Leaves | Stems | Leaves | Stems | Leaves |
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| Plainfield..... | 36.1 | 63.9 | 40.5 | 59.5 | 64.1 | 35.9 | 55.6 | 44.4 |
| Kewanee..... | 40.9 | 59.1 | 45.5 | 54.5 | 60.4 | 39.6 | | |
| Onaway..... | 39.1 | 60.9 | | | 56.4 | 43.6 | 57.2 | 42.8 |
| Hillsdale..... | 42.6 | 57.4 | 45.0 | 55.0 | 55.6 | 44.4 | 53.8 | 46.2 |
| Brookston..... | 40.0 | 60.0 | 33.3 | 66.7 | 56.5 | 43.5 | | |
| Miami..... | 40.4 | 59.6 | 41.7 | 58.3 | 56.5 | 43.5 | | |

SUMMARY AND CONCLUSIONS

In this work the influence of the growth of field peas upon the calcium and magnesium content of the soil solution and upon the reaction of the soil was observed. Likewise, variations in the calcium and magnesium content of pea stems and leaves and in their juice when obtained from plants grown on different soil types were studied. The relationship of certain characteristics of the different soil types to the amounts of calcium and magnesium present in the pea plants was considered.

Growing pea plants greatly reduced the amounts of calcium and magnesium present in the different soil solutions. They also tended to decrease the acidity of strongly acid soils and to increase that of alkaline or nearly neutral soils.

The calcium and magnesium content varied greatly in the pea plants grown on the different soil types. On soils of similar texture and reaction, the amount of calcium present in the pea plant varied directly with the amount

present in the soil solution. Soil texture and soil reaction influenced the calcium content of the peas to the extent of obscuring the effect of the concentration of the soil solution if not allowed for. The magnesium content of the plants was very irregular and appeared to be influenced more by soil texture than any other characteristic of the soils studied here.

There was more calcium and magnesium present in the tissue and in the juice of pea leaves than in the tissue and the juice of the stems. As the plants became older, the concentration of calcium and magnesium increased in the tissue and juice of pea leaves, whereas it sometimes increased and sometimes decreased in the tissue and juice of stems.

Greater amounts of calcium than of magnesium were always present in the tissue and in the juice of pea stems and leaves.

It appeared that the calcium and magnesium content was higher in slowly growing plants than in those making a rapid growth.

The proportion of stems was smaller than that of leaves in the young plants but became greater as the plants advanced in growth.

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THE ULTIMATE NATURAL STRUCTURE OF SOILS

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The texture of soils is usually defined as the ultimate size of soil particles, and structure of soils is commonly defined as the arrangement of these soil particles under actual soil conditions. This arrangement or structure assumes all sorts of forms under actual soil conditions, depending largely upon the soil type and treatment. It may be in the form of single grained particles, compound particles or granules, large and small blocks or clods.

The structure of soils as found under field conditions is not the ultimate and natural structure. This kind of structure is dependent on, and is the result of, many external factors, such as degree of moisture at which soil is worked, whether the soil has been puddled or not, and amount of work or degree of pressure applied in obtaining a proper tilth. In other words, this structure is only a temporary, accidental, artificial, and changeable condition.

Soils, however, appear to have a natural, ultimate, and stable structural condition. That is to say, the molecular, cohesive, adhesive, and cementing forces and the type tend to produce or give a soil a structure that is naturally and ultimately stable or more or less permanent and requires external energy or force to destroy it. For instance, in dispersing soils for mechanical analysis, it is this stable, ultimate, natural structure that is being destroyed, and as is well known, in some soils it is very hard to destroy it even with the application of an enormous amount of external energy.

This natural, ultimate, and stable structural condition of soils seems never to have been measured or studied; and the main reason for it is undoubtedly the lack of methods.

In 1924 (1) and also in 1927 (2) work was presented showing that when soils in the dry condition are placed in an excess of water they tend to disintegrate or slake into single or compound particles of various sizes. It was also revealed that on account of this principle, water plays a tremendously important rôle in producing and keeping a good tilth in soils.

An experimental test of the hydrometer method, developed for studying the texture of soils (3), for the study of the ultimate natural structure of soils seems to have proved successful.

It was found that when soils in dry condition were placed in an excess of water they would immediately begin to disintegrate or slake into particles or granules of various sizes. These particles or granules seemed to be in an ultimate natural size and stable condition, because according to the hydrometer method they would not get smaller upon standing in water for an indefinite length of time, nor upon gentle shaking. Indeed, allowing the soils to stay in

water for five days, or shaking them by hand in a liter cylinder from 6 to 30 times produced results that remained quite constant or changed very little. It would take very long and vigorous shaking, or the application of a considerable amount of energy, to break up these particles or granules from their natural, stable condition.

It seems quite certain, therefore, that soils have a natural, ultimate stable structure which is definite for any one soil, and that the phenomenon of slaking presents a natural and logical means of measuring accurately this structure. Indeed, it now appears that slaking is a fundamental phenomenon and the results it yields in soils with water are definite in character. That is to say, soils disintegrate or undergo a mechanical dissolution, somewhat similar to a chemical dissolution, into their natural, ultimate structure, which is stable and can only be destroyed by the application of external energy. This mechanical dissolution of soils is brought about by the attraction of the soil particles for water, and the film of water intervening or surrounding the particles destroys their force of cohesion or attraction for each other.

It is the object of this paper, therefore, to present data on the measurement of the natural ultimate structure of the various types of soils, by means of the phenomenon of slaking and the hydrometer method. In other words, it is the measurement of the texture of the natural ultimate structure of soils.

METHOD AND PROCEDURE

In order to facilitate the rate, insure completeness of slaking, and eliminate any mechanical handling which would tend to cause artificial dispersion, a suitable method had to be devised. The method that was worked out and finally adopted consisted of placing the soil into a bag made of 2-mm. window screen and suspended in the special 1000-cc. hydrometer cylinders filled with water to the proper mark (3). As soon as the soil was dropped into the wire bag and came in contact with the water it would immediately begin to slake into particles or granules of various sizes, and these particles would go through the 2-mm. wire screen and fall into the mass of water or to the bottom of the cylinder. To prevent the particles or granules from accumulating in the bag as they slaked from the mass of soil, the bag was pushed gently up and down in the water occasionally and the slaked portion of the soil would immediately fall into the water. As the slaked portion of the soil would leave the bag, more surface of the unslaked soil would be exposed, which in turn would slake at a greater rate. The bag would be left in the water with occasional gentle pushing up and down, until all the soil had slaked and fallen out of the bag. To assure complete slaking, however, the soils would be left in the water at least two hours.

The use of the wire bag was helpful because (a) it prevented the shielding effect of the slaked portion of the soil in the unslaked portion, (b) the rate of slaking was thereby considerably hastened, and (c) it showed definitely when the soil was completely slaked.

Since it was the object of this investigation to study the natural ultimate

structure of soils, only soils in the natural field conditions were used. That is to say, great care was taken not to pulverize the soil in any way but to use it just as it would exist in the ground.

On the other hand, it was found that the phenomenon of slaking requires that the soil be dry. In other words, unless a soil is in a dry state it will not break down in water into its ultimate natural structure. This may be attributed to the fact that the phenomenon of slaking is brought about by the attraction of the soil particles for water, and the attractive forces for water are greatest only when the soil is dry. Hence, all the soils that were used in this investigation were in an air-dry condition.

The amount of air-dry soil employed was equivalent to 50 gm. on an oven-dry basis, in every case. This weighed amount of soil, would be dropped, as previously stated, into the wire bag suspended in water in the cylinder and allowed to slake. Any gravel that remained behind would be weighed and taken into consideration in the final calculation of the results.

After a soil had completely slaked, 5 cc. *N* KOH was added and then it was shaken six times by placing one palm on the mouth of the cylinder and turning the latter completely upside down and back three times. Then the cylinder was placed on a table, the time being noted immediately, the hydrometer put in the cylinder, and readings taken at the end of 10, 30, and 60 seconds and also at the end of 15 minutes.

It may seem that it is almost impossible to take hydrometer readings at the end of 10 seconds. It is true that it is a somewhat difficult test, but after a little experience it can be done rather easily. There is no difficulty, however, in taking readings at the end of 30 seconds.

The hydrometer readings are then translated directly into size of particles. This is accomplished by the aid of Stoke's law as shown in a previous communication (3). According to Stoke's law, and basing the calculations on a column of liquid $32\frac{1}{2}$ cm. high and a temperature 20°C ., the sizes of particles that will fall or still stay in suspension at the various times are as follows:

| <i>Time of sedimentation</i> | <i>Diameter of particles</i> <i>mm.</i> | <i>Time of sedimentation</i> | <i>Diameter of particles</i> <i>mm.</i> |
|------------------------------|--|------------------------------|--|
| 10 seconds | 0.1914 | 11 hours | 0.00303 |
| 30 seconds | 0.11009 | 12 hours | 0.0029 |
| 60 seconds | 0.07785 | 13 hours | 0.00279 |
| 2 minutes | 0.055050 | 14 hours | 0.00270 |
| 5 minutes | 0.034814 | 15 hours | 0.00259 |
| 15 minutes | 0.02010 | 16 hours | 0.0025 |
| 30 minutes | 0.014212 | 17 hours | 0.00243 |
| 60 minutes | 0.01005 | 18 hours | 0.002369 |
| 2 hours | 0.0071 | 19 hours | 0.002307 |
| 3 hours | 0.0056 | 20 hours | 0.00223 |
| 4 hours | 0.00502 | 21 hours | 0.00219 |
| 5 hours | 0.0045 | 22 hours | 0.00214 |
| 6 hours | 0.0041 | 23 hours | 0.00209 |
| 7 hours | 0.0038 | 24 hours | 0.00205 |
| 8 hours | 0.00355 | 48 hours | 0.00145 |
| 9 hours | 0.00335 | 96 hours | 0.001025 |
| 10 hours | 0.00318 | | |

The foregoing figures for size of particles signify the upper limit of size of any particle that will stay in suspension at any given time. For instance, at the end of one minute the upper limit of any particle that may still stay in suspension is 0.077846 mm. in diameter, at the end of one hour 0.01005 mm., etc.

In the present investigation, however, the hydrometer readings were taken at only four different periods, after 10, 30, and 60 seconds, and after 15 minutes. Consequently the largest particles, or upper size limit of particles that would still be in suspension would be 0.1914, 0.11009, 0.07785, and 0.02010 mm., respectively.

It is at once seen that the largest size of particles that can be measured by the hydrometer and Stoke's law is about 0.2 mm., which is the border-line between medium and fine sand. It is unfortunate that particles larger than the above cannot be measured by this combined method. But even with this limitation the method yields much information concerning the ultimate natural structure of soils, which is of great significance, as will be seen.

In order to get a comparison between the ultimate natural structure and the ultimate texture of soils, the same sample of soil that was used to measure the structure was dispersed by the special soil dispersing machine, previously described (3) and hydrometer readings were taken at the same periods. It was believed that such a comparison would possess distinct value. For instance, if a soil when dispersed shows that it contains 85 per cent of clay and before dispersing shows that it contains only 3 per cent of clay, then it must be assumed that nearly all the clay portion of this soil exists, or slakes naturally, in particles or granules much larger than the clay size. If, on the other hand, a soil when dispersed shows that it contains 25 per cent clay and before dispersion 11 per cent clay, then it follows that a large portion of the clay of this soil exists, or slakes naturally, in particles of the clay size and a comparatively smaller portion in larger particles or granules.

It might be thought that the addition of potassium hydroxide would affect the results. As stated in previous paper (3), however, the main function of the presence of the potassium hydroxide seems to be to stabilize the soil suspension rather than to increase dispersion.

EXPERIMENTAL RESULTS

Before the actual experimental data are presented on the ultimate natural structure of soils, it is necessary to present first results showing that when a soil slakes in water it slakes into particles, or granules, which are rather surprisingly stable and do not easily become smaller when gently shaken or when allowed to remain in water for an indefinite length of time. It is really this rather striking stability that makes it possible to measure the natural ultimate structure of soils.

The results given in table 1 are typical of a large number of soils examined repeatedly. As previously stated, these results were obtained by allowing the soil to slake completely in the cylinder, and then placing one palm on the

mouth of the cylinder, and turning the latter completely upside down and back three times and then taking the hydrometer readings. This form of shaking would be repeated many times.

TABLE 1

Stability or the resistance of the naturally slaked particles or granules of soil to become smaller when shaken gently or allowed to stand in water

(Results show percentage of soil material still in suspension whose upper limit is indicated at each period)

| | TIME AND DIAMETER | | | |
|--------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | 10 seconds, 0.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. |
| | per cent | per cent | per cent | per cent |
| <i>Michigan clay</i> | | | | |
| Shaken 6 times..... | 25.0 | 12.0 | 8.0 | 3.0 |
| Shaken 12 times..... | 25.0 | 12.5 | 9.0 | 3.1 |
| Shaken 18 times..... | 26.0 | 13.8 | 9.4 | 3.4 |
| Shaken 24 times..... | 26.0 | 14.0 | 10.0 | 3.4 |
| Shaken 30 times..... | 26.0 | 14.0 | 11.0 | 3.5 |
| Completely dispersed..... | 88.5 | 86.3 | 84.8 | 83.6 |
| <i>Missouri silty clay</i> | | | | |
| Shaken 6 times..... | 41.0 | 25.0 | 19.0 | 4.0 |
| Shaken 12 times..... | 41.0 | 27.0 | 20.0 | 4.0 |
| Shaken 18 times..... | 41.0 | 28.0 | 22.0 | 4.5 |
| Shaken 24 times..... | 41.0 | 28.5 | 23.0 | 5.2 |
| Shaken 30 times..... | 42.0 | 29.0 | 24.0 | 6.0 |
| Completely dispersed..... | 87.5 | 82.4 | 80.0 | 59.7 |
| <i>Michigan clay</i> | | | | |
| Stood 1 hour, shaken 6 times..... | 26.8 | 14.3 | 11.6 | 3.8 |
| Stood 24 hours, shaken 12 times..... | 26.8 | 14.7 | 12.1 | 4.1 |
| Stood 48 hours, shaken 18 times..... | 27.3 | 14.2 | 12.7 | 3.9 |
| Completely dispersed..... | 88.5 | 86.3 | 84.8 | 83.6 |
| <i>Missouri silty clay</i> | | | | |
| Stood 1 hour, shaken 6 times..... | 41.8 | 26.1 | 18.8 | 4.2 |
| Stood 24 hours, shaken 12 times..... | 42.5 | 27.3 | 21.1 | 4.2 |
| Stood 48 hours, shaken 18 times..... | 43.1 | 28.2 | 22.6 | 4.9 |
| Completely dispersed..... | 87.5 | 82.2 | 80.0 | 59.7 |

It is at once apparent, therefore, that when soils slake in an excess of water into particles and granules, these particles and granules are stable and in equilibrium, and neither gentle shaking nor long standing in water will decrease their size. Hence, they must represent the ultimate, natural structure

of soils. Some soils that slake with great difficulty may show greater increase of dispersion with greater number of times of shaking than is shown in the above soils.

The term "gentle shaking" is used. This term, however, is only relative. The shaking is gentle in comparison to the dispersing machine, wherein the soil particles are whirled around at a speed of over 10,000 revolutions per minute, but it is quite vigorous when it is considered that a soil is shaken 6 to

TABLE 2

Percentage of soil material still in suspension whose upper limit of size is indicated at each period
(Results are intended to indicate the mechanical analysis of the ultimate natural structure of soils as compared to their ultimate texture or mechanical analysis)

| | NATURALLY SLAKED | | | | MECHANICALLY DISPERSED | | | |
|--|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | Time and diameter | | | | | | | |
| | 10 seconds, 1.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. | 10 seconds, 0.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. |
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| 1. Fresno fine sandy loam..... | 48.0 | 33.0 | 25.0 | 5.6 | 46.0 | 38.0 | 30.0 | 11.10 |
| 2. Susquehanna fine sandy loam..... | 35.0 | 18.5 | 14.5 | 5.0 | 40.0 | 26.0 | 18.0 | 10.00 |
| 3. Portsmouth sandy loam..... | 28.0 | 17.0 | 12.0 | 3.0 | 40.0 | 28.0 | 22.0 | 8.4 |
| 4. Hagerstown loam..... | 17.0 | 10.0 | 7.5 | 2.3 | 75.0 | 70.0 | 67.5 | 48.5 |
| 5. Michigan silt loam..... | 51.0 | 38.0 | 34.0 | 14.0 | 58.0 | 54.0 | 50.0 | 17.4 |
| 6. Grundy silt loam..... | 68.0 | 50.0 | 38.5 | 5.0 | 88.9 | 85.0 | 76.0 | 34.0 |
| 7. Antrim county silt clay..... | 38.5 | 27.0 | 23.5 | 8.0 | 74.8 | 72.0 | 68.0 | 45.0 |
| 8. Brookston silt loam (surface)..... | 32.0 | 19.0 | 14.0 | 5.0 | 51.0 | 43.0 | 39.0 | 25.5 |
| 9. Miami silt loam (0-6 inches)..... | 38.0 | 24.0 | 21.0 | 7.0 | 56.0 | 45.0 | 41.0 | 22.0 |
| 10. Napanee silt loam (0-6 inches)..... | 38.0 | 26.0 | 22.0 | 11.0 | 58.2 | 54.0 | 53.0 | 38.0 |
| 11. Ontonagon silt loam (0-6 inches).... | 56.0 | 41.0 | 30.0 | 9.0 | 86.0 | 82.0 | 75.0 | 54.0 |
| 12. Minn Clyde silt loam..... | 46.0 | 28.0 | 13.0 | 8.0 | 79.6 | 76.0 | 72.0 | 35.0 |
| 13. Miami silt loam C (40-60 inches).... | 50.0 | 30.0 | 13.0 | 9.0 | 80.3 | 76.0 | 72.0 | 52.0 |
| 14. Miami silt loam B (8-30 inches)..... | 17.0 | 13.0 | 11.0 | 4.5 | 65.3 | 62.0 | 59.0 | 40.0 |
| 15. Susquehanna clay C..... | 28.0 | 14.0 | 9.0 | 4.5 | 71.0 | 66.0 | 63.0 | 58.0 |
| 16. Residual limestone clay..... | 26.0 | 13.0 | 8.5 | 3.3 | 88.0 | 86.0 | 85.0 | 83.0 |
| 17. Alkali soil..... | 52.0 | 34.0 | 24.0 | 7.5 | 56.0 | 37.0 | 28.0 | 18.5 |
| 18. Putman silty clay B..... | 42.3 | 26.2 | 18.8 | 5.3 | 88.6 | 83.1 | 80.6 | 60.1 |

30 times by allowing it to fall each time a distance of over 16½ inches in the cylinder.

The final results are shown in table 2.

A critical examination of the results in table 2 reveals many interesting and significant facts regarding the ultimate natural structure of soils, as well as their ultimate texture. A comparison of the two columns under the 15-minute period readily demonstrates that when soils slake, they have comparatively

little material under 0.0201 mm. This is true not only with the sandy loams and loams but even with the heaviest clays. For instance soil no. 16 when dispersed contains 83.0 per cent material under 0.0201 mm. whereas before it is dispersed, or when it naturally slakes, it has only 3.3 per cent under the same size of particles. The amount of soil material under 0.0201 mm. is less than 10 per cent in nearly all the soils when they are allowed to slake, but when they are dispersed the amount of material under this size increases from about $1\frac{1}{2}$ times to more than 25 times. Hence the texture of the ultimate natural structure of soils is mainly coarse.

In this connection it is very interesting and important to note that sandy loams and loams which have a relatively small amount of total clay have relatively and in many cases, absolutely, more fine slaked material than the clays and clay loams which possess a very high clay or colloidal content. For instance soil 2 contains only 10 per cent total material under 0.0201 mm., whereas in the slaked condition it contains 5 per cent. On the other hand soils 11, 13, 15, and 16 contain 54, 52, 58, and 83 per cent total material respectively under 0.0201 mm., but in the slaked condition they contain only 9, 9, 4.5, and 3.3 per cent, respectively, of the same fine material.

This same kind of difference seems to exist also between surface and sub-surface soils. As a rule, surface soils seem to have somewhat more fine material in the slaked condition than the subsoils, even though the latter may contain considerably more total clay or colloids. For instance, surface soils 9 and 10 contain 7 and 11 per cent material under 0.0201 mm. and they contain 22 and 38 per cent, respectively, of total material of the same size while many of the subsoils which contain 60 and 80 per cent of the fine material do not contain any more of the same fine material in the slaked condition. Indeed, many of the substrate soils as Miami Silt loam B tend to slake into large pieces.

A comparison next of the results in the two columns under the 10-second period, also show some very interesting and significant contrasts in the various types of soil. One of the most significant things brought out is the fact that clay soils, and especially those of the deeper horizons, have more particles or granules larger than 0.1914 mm. than do the light types of soils, which contain only a moderate or very little total clay content. For instance, soil 1 which contains only 11 per cent material under 0.0201 mm. contains 48 per cent under 0.1914 mm. or 52 per cent above this size; whereas soil 4 which contains 48.5 per cent material under 0.0201 mm. has only 17 per cent under 0.1914 mm., or 83 per cent above this size. Similar differences are seen in most of the other soils. Apparently, therefore, the more clay a soil contains, and the purer this clay is, the greater, as a rule, is its tendency to slake or disintegrate into larger particles or granules, or pieces. This may be attributed to the greater cohesive force, for like has greater attraction for like.

A comparison of the results in the respective columns under the 30- and 60-second period shows the same tendencies for the various soils, with slight

decrease of variations, as those revealed in the columns under the 10-second and 15-minute periods, which are the extremes.

At this point it would be proper to ask whether the ultimate natural structure of soils when destroyed by dispersion or puddling, remains permanently in that destroyed condition or whether it is in time restored. That is to say, if a soil is dispersed by the machine, so that its particles are reduced to their ultimate size, will it regain its former or original structure after it is dried again, or will it remain indefinitely in the dispersed condition.

The answer to this question is given by the results in table 3. These results were obtained by allowing the soils to slake naturally. Their mechanical analysis was then ascertained by the hydrometer method already described. These naturally slaked soils were then dispersed by the special dispersing machine and their mechanical analysis was again measured. The dispersed soils were then poured into large beakers and evaporated to dryness. The dried soils were wetted and dried at least once or twice. These dispersed soils in the dry condition were then dropped into the wire bag suspended in water in the cylinder and allowed to slake naturally. After the soils had completely slaked they were shaken 6 to 10 times and their mechanical analysis was determined.

Examining first the results in the three columns under the 15-minute period (table 3) we see at once that the dispersed soils tend to go back to their original natural structure by the process of drying and wetting. This is revealed by the fact that the amount of the fine material under 0.0201 mm. is smaller in the slaked dispersed soils than in the mechanically dispersed soils, and tends to approach that in the naturally slaked soils. For instance, soil 1 has 83 per cent of fine material under 0.0201 mm. when mechanically dispersed, but it has only 5.5 per cent of the same size when this dispersed soil is dried, wetted, and slaked. While in the naturally slaked condition, this soil has 3.3 per cent of the same fine material. Soil 6 has 35 per cent of its material under 0.0201 mm. when mechanically dispersed, 13.5 per cent when the mechanically dispersed soil is slaked, and 8 per cent when the natural soil is slaked.

Apparently, therefore, when the natural structure of a soil is destroyed and reduced to the ultimate size particles, this condition is not stable or in equilibrium and upon drying, these ultimate size particles tend to unite and go back to the natural, ultimate structure, which is stable and in equilibrium. The ability of the ultimate size particles to go back to the natural structural condition, varies with the soil. With some soils, they go back at once, as in soil 1; with other soils they go back more slowly, and they require a larger number of times of wetting and drying to do it.

Examining next the results in the various columns under the 10-, 30-, and 60-second periods, we note that the amount of material under 0.1914, 0.1101, and 0.0778 mm., is considerably greater in the slaked, dispersed soils than in the natural, slaked soils. Apparently, particles of this size in the dispersed soils do not tend to go back to their original structure as easily as those below

0.0201 mm. It must be remembered, however, that the larger sizes represent separated sands, which would not be expected to form larger particles or granules unless they were cemented or united by the finer particles. Hence, these results are just what should be expected. Under further wetting and drying and thoroughly mixing, they too would ultimately go back to the original structure or to that shown when natural soils are slaked.

Soil 10 in table 3 merits special attention. It is composed entirely of pure

TABLE 3

Effect of drying and wetting on the structure and texture of soils which had been mechanically dispersed

(Results indicate percentage of soil material still in suspension whose upper limit of size is indicated by each period)

| | NATURAL SOIL SLAKED | | | | NATURAL SOIL MECHANICALLY DISPERSED | | | | DISPERSED SOIL EVAPORATED TO DRYNESS, WETTED AND DRIED, AND ALLOWED TO SLAKE | | | |
|---|---------------------------|---------------------------|---------------------------|---------------------------|--|---------------------------|---------------------------|---------------------------|--|---------------------------|---------------------------|---------------------------|
| | Time and diameter | | | | | | | | | | | |
| | 10 seconds, 0.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. | 10 seconds, 0.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. | 10 seconds, 0.1914 mm. | 30 seconds, 0.1101 mm. | 60 seconds, 0.0778 mm. | 15 minutes, 0.0201 mm. |
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| 1. Residual limestone clay..... | 26.0 | 13.0 | 8.5 | 3.3 | 88.0 | 86.0 | 85.0 | 83.0 | 65.0 | 42.0 | 32.0 | 5.5 |
| 2. Susquehanna clay C. | 28.0 | 14.0 | 9.0 | 4.5 | 71.0 | 66.0 | 63.0 | 58.0 | 65.5 | 52.0 | 45.0 | 22.0 |
| 3. Naponee silt loam (0-6 inches)..... | 38.0 | 26.0 | 22.0 | 11.0 | 58.2 | 54.0 | 53.0 | 38.0 | 38.0 | 26.0 | 21.0 | 14.0 |
| 4. Ontonagon silt loam (0-6 inches)..... | 56.0 | 41.0 | 30.0 | 9.0 | 86.0 | 82.0 | 75.0 | 54.0 | 65.8 | 56.5 | 47.5 | 21.5 |
| 5. Hagerstown loam... | 17.0 | 10.0 | 7.5 | 2.3 | 75.0 | 70.0 | 67.5 | 48.5 | 66.0 | 51.0 | 44.5 | 17.5 |
| 6. Minn clyde silt loam.. | 46.0 | 28.0 | 13.0 | 8.0 | 79.6 | 76.0 | 72.0 | 35.0 | 71.0 | 61.8 | 49.5 | 13.5 |
| 7. Miami silt loam B... | 17.0 | 13.0 | 11.0 | 4.5 | 65.3 | 62.0 | 59.0 | 4.0 | 64.5 | 63.5 | 44.5 | 22.5 |
| 8. Brookston silt loam (0-6 inches)..... | 32.0 | 19.0 | 14.0 | 5.0 | 51.0 | 43.0 | 39.0 | 25.5 | 36.8 | 27.0 | 24.0 | 8.0 |
| 9. Michigan silt loam... | 51.0 | 38.0 | 34.0 | 14.0 | 58.0 | 54.0 | 50.0 | 17.4 | 55.0 | 43.0 | 38.5 | 8.9 |
| 10. Pure colloids from soil 2..... | | | | | 100.0 | 100.0 | 100.0 | 100.0 | 7.0 | 5.0 | 4.5 | 3.5 |

colloids which were extracted by the process of decantation from soil 15 in table 2. These pure colloids were evaporated to dryness and then allowed to slake, as in the case of the other soils. These colloids when in dispersed condition gave 100 per cent colloids in suspension by the hydrometer method. Now, when these colloids were evaporated to dryness and then allowed to slake, they did not slake into fine particles again but rather into medium and large flat pieces, which would not pass the 2-mm. wire bag; and, as shown in the table, hardly any material stayed in suspension.

The refusal of these pure soil colloids to slake again into fine particles is a representative phenomenon in soils. It goes to support the statement already made, that the higher and purer is the clay content in soil, the coarser is the structure into which it slakes. This might be attributed to the fact that the cohesive, adhesive, and attractive forces are greater in such materials and these forces prevent them from slaking into very fine particles.

The tendency of the pure colloids as well as the highest clay content soils and subsoils to slake into coarser material than do the lighter textured soils, is in conformity with the behavior of these soils under field conditions. For instance, it is common knowledge that it is almost impossible to work certain clay soils and subsoils of very high clay content into a fine tilth. On account of the great adhesive, cohesive, attractive, and cementing forces, these soils have a predominant tendency to break into clods. On the other hand, light textured soils have a predominant tendency to break into rather fine textural tilth. Hence, the experimental results here obtained have a close relation to practical experiences, and careful judgment has to be employed in interpreting them.

Since the structure into which soils naturally slake is the ultimate, natural, and stable structure, then it would seem that under field conditions the natural tendency of soils would be to maintain this structure. Of its own accord, this structure would not get finer or coarser unless it was brought about by some external agent, such as the use of implements in the preparation of the seedbed. But even then, this change of the natural, ultimate structure would be temporary, for the process of wetting and drying would tend to bring it back to its original natural and stable condition.

Under field conditions the natural, ultimate structure of soils may or may not be seen. What is usually seen is the accidental, artificial, and changeable structure.

Finally, it must be emphasized (*a*) that on account of the tendency of soils to slake into their natural, ultimate structure, (*b*) because this natural, ultimate structure tends to be stable, and (*c*) since the soil particles have such a strong tendency to go back to the natural, ultimate structure when it is once destroyed, the work previously reported (1), that water is the greatest single agent in producing and maintaining soil granulation, is well confirmed and supported by the present findings.

SUMMARY

When soils in the natural state but dry condition are placed in an excess of water they slake or disintegrate into particles and granules of various sizes.

Slaking these particles and granules in a large quantity of water, gently but quite vigorously, does not decrease their original size or slaked condition. To make them smaller or break them up further, a large amount of external energy or force has to be applied.

The size into which the particles and granules slake naturally, seems to be remarkably stable and in equilibrium, and a large amount of force has to be applied to make this original size smaller.

Since the size of the particles and granules into which soils slake in water has such marked stability, it is believed that these particles and granules constitute the natural, ultimate structure of soils. In other words, when natural, dry soils are placed in excess of water, they slake into their ultimate, natural structure.

The ordinary structure that is seen under field conditions is not the natural, ultimate structure. This field structure is the accidental, artificial, changeable, and temporary structure.

By means of the hydrometer method it is possible to make a mechanical analysis of this natural, ultimate structure and thereby ascertain the size and proportion of the various particles and granules. In other words, it seems that it is as possible now to determine the texture of the natural, ultimate structure of soils as it is to determine the ultimate size of particles.

The experimental data presented would seem to support those views.

It would appear that this natural ultimate structure of soils ought to form the basis for the study of many soil physical properties such as percolation, penetration, etc.

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SOIL PROFILE STUDIES: I. SOIL AS AN INDEPENDENT BODY AND SOIL MORPHOLOGY¹

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The study of soils began not as an independent branch of science, but as an adjunct to some established scientific discipline, such as geography, geology, mineralogy, chemistry, or a combination of some of these. The scientific study of soils began in the laboratory, not in the field. As a result, soil morphology made no progress until extensive studies in the field forced the workers to describe the soil, give its morphological characters, such as color, structure, constitution, consistency, and texture. But in the early period of field study, it was not morphology of soils, but of soil material. Not until the soil was recognized as an independent natural body did scientific soil morphology find its place in the scheme of soil studies and became the valuable aid in unravelling the problems connected with soils.

HISTORICAL

Soil science as an independent science

Because of its geographic position Russia presented an ideal geographic unit for the systematic study of soils. The vast stretches of the plain in European Russia with its fairly homogeneous character of topography at its gradual change of climatic conditions, expressed by the temperature and moisture relationships, as one moves from north to south made it imperative for the man in the field to study the soil in all its aspects. The consequence of this favorable physico-geographical position was that the Russian soil workers were the first to recognize the soil as a distinct and independent discipline of natural science. One may find hints to that effect in the work of other students of soils long before the savant and founder of the Russian soil science, Dokuchaev, announced his conclusions on the genesis of the Russian chernozem (black earth), which served as the basis for ushering in the new concept of soils as a historical, independent, natural body.

The natural scientists and early students of soils did not look upon soils as a distinct branch of natural science. Soils were appreciated simply as an object of agricultural activities for the human. The famous chemist, Berzelius,

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calls the soil "the chemical laboratory of nature in whose bosom various chemical decompositions and syntheses take place in large quantities in a hidden manner." [Quoted from Yarilov (77).] Sprengel (62) designates the soil as a changed mass of material derived from minerals containing the decomposition products of plants and animals.

Thaer (66) looked upon soils from a utilitarian standpoint. He divided soils into six species, each one being subdivided into several classes primarily on the basis of their utility. The species are: (a) Clay soils, (b) loam soils, (c) sandy loam and loamy sand, (d) sandy soils, (e) humus soils, and (f) limestone soils. This is a purely physical concept. Thaer calls the soil "a raw material from which the agriculturist obtains various organic products without which he could not persist."

The geologic point of view was developed by Berendt (5). He states: "Petrography and pedography, the study of native rocks, and soil science are branches of the same science—geognosy." He distinguishes between "Boden" and "Grund." The latter according to Berendt is "the native rock which appears to us in undisturbed solid form." The former is considered as "the part of native rock which comes out to the surface and which is mellowed mechanically because of its contact with the air, which changes it chemically."

The famous German soils man, Wahnschafte (71), does not agree with Berendt's definition of soils, which would exclude the marsh and peat soils. He therefore gives his own definition. "Soil is not a geognostic conception, but a cultural-technical, primarily an agricultural. . . . As soils I understand the upper mellow and earthy layer of the earth's mantle even though it may support but the poorest vegetation."

Fallou (16) occupies a unique place in the history of soil science. His work has not been appreciated and has been forgotten, although historically he may be looked upon as the founder of pedology. Fallou showed how the utility standpoint of the students of soils, up to his time, prevented the crystallization of a scientific appreciation of the nature of soils as such. He criticized severely the chemical theory of soils. He wrote: "Recently the millenium for agriculture was looked for from the chemist; it was thought that a chemical analysis of the soil would give a complete idea about the soil. . . . Soil science was recognized not as a science by itself, but as a branch of agricultural chemistry. . . . Soil science is an empirical science. Nature itself is its source. Observations on soils in their geognostic relations, or in their relation to the strata formation and to the underlying rock are of special importance." We may readily see that Fallou was a proponent of the purely geognostic or geologic point of view. He realized, however, that the science of geology does not exclude soil science as a distinct discipline. "Just as petrification is looked upon independently of the native rock which accompanies it and we have paleontology as a distinct science, in the same way, soils may be separated from the native rocks and investigated as a separate independent scientific discipline." The definition given by Fallou for soils is: "Soil is

decomposed, more or less disintegrated native rock distinct and separate from the compact, undisturbed native rock, with an admixture of organic materials; the rock has changed and metamorphosed in its form and infrequently also in its makeup. Soil as such does not therefore belong any more to the rock formation, but is a formation by itself."

Contemporaneously with Fallou, Dokuchaev began to develop his views on soils as a result of his extensive studies of the great belt of black earth found in Russia, known as chernozem.

Fallou, as has been pointed out, had recognized the soil as a distinct natural body, but he presented no evidence to that effect and for this reason his presentation of the soil classification did not stand the test and found no support in later years.

Richthofen (51), who followed in the footsteps of Fallou, did not differentiate sharply between soils and powdered and crushed rock materials obtained mechanically. Instead of connecting the regional distribution of soils with the physico-geographical conditions responsible for the dynamic processes, he fell back on the geological periods. And Glinka (19) justly states: "When one speaks of geographical position he understands the existence of a natural relation between the present distribution of climatic elements and the present geography of the soil cover. The regionality as described by Richthofen has at times no connection with the present climatic conditions. Thus the regions of glacial denudation, accumulation, river denudation, abrasions, and of volcanic transport exist on the surface of the earth entirely independently of the present climatic conditions." Richthofen, as well as Walther (72) who continued to develop the ideas of the former (51) considered the distribution of soils not from the standpoint of their origin but from their position. For this reason the "soil as natural historical body" was interchangeable in their scheme with geological material of soils, which eventually would be converted into soil as a natural body. It is this point which distinguishes Dokuchaev's views as a unique contribution which was later developed by other Russian soil scientists.

Dokuchaev as a trained geologist started out with the geologic point of view on soils current in those days. As soon as he came in contact with the vast stretches of Russian chernozem his keen eye immediately noted the homogeneous character and features (morphology) of the soils in a definite geographic region. In 1877 (10) he stated: "Whether we admit that the southwestern portion of Russia was submerged under the sea in the beginning of the post-tertiary period, as some geologists think, or it was covered by glaciers, as other geologists think, or it was dry land, as still another group of geologists think, matters little. For us it is important that after this or the other of the given phenomena the upper layers of the soils were apparently subject to various processes due to weathering and to processes due to vegetation; both of these were instrumental in changing the upper horizon of the parent material to a greater or lesser depth. These parent materials which have undergone

changes by the mutual activities of air, water, and plants, I call soil." In 1879 (11, 12) Dokuchaev formulated his ideas on soils in general: "Soils are the superficial mineral and organic constituents, always more or less colored by the humus, which constantly manifest themselves as a result of the combined activity of the following agencies: living and dead organisms (plants and animals), parent material, climate, and relief."

The original views of Dokuchaev differ little from those of the Western European students of soils and of Hilgard in the United States, who appreciated the genetic relationships in soil formation. The genetic principle as the foundation of soil classification was known and used by other workers besides Dokuchaev. Thus Hilgard (21, 22) in his extensive studies of the soils of the United States could not help but notice the regularity of the distribution of soils under the various physico-geographical conditions of the country. Hilgard had therefore, the genetic approach, noting the relations of the various soils with the different natural conditions and factors of soil formation. He failed, however, to see soils in their morphologic constitution as a result of the soil forming processes. He appreciated the factors of soil formation, but failed to correlate them and build a system of soil classification based on the correlated factors. Of the factors of soil formation Hilgard emphasized the moisture factor.

In discussing the relations of soils to climate Hilgard (21) said: "Since soils are the residual product of the action of meteorological agencies upon rocks, it is obvious that there must exist a more or less intimate relation between the soils of a region and the climatic conditions that prevail, or have prevailed therein."

"Since water is the prominent agent in soil formation, it follows that the variations in its supply—in other words, the greater or less amount of rainfall—must affect materially that process." The logical consequence of such a viewpoint was Hilgard's broad division of soils into two large groups: arid and humid.

Fundamentally the parent rock was the starting point of Hilgard's elucidation on soil characters and features, and in this respect he may be considered as an adherent of the geologic school of soil science.

The geologic point of view predominated in the work of the other early American students of soils. Thus Shaler (54) in his splendid monograph treats the subject of soils from the geologic point of view. To him soil is "a mixture of decayed rock and organic matter." Johnson (25) simply states that "soils are broken and decomposed rock." King (26), one of the keenest of American soil students, also had the geologic point of view.

There is a lot of material on the history of the subject in the reports of the geological surveys of the states and in the reports of the different agricultural societies in the United States. A partial list of references may be found in No. 13 Bibliographical contributions, U. S. Department, Agriculture Library, published in 1927.

The far reaching effects of Dokuchaev's later views (13, 14) consisted "*in excluding soils from the system of surface cover formations and placing them into a distinct independent system of natural science.*" [Quoted from Afanasiev (2).] For Dokuchaev soil science is just as distinct a science as botany, zoology, or any other of the natural sciences. This view was an outgrowth of his original thesis that "soil is an independent, natural, historical body." The factors of soil formation determine the type of soil in its genetic construction as manifested in the profile. "If we know the factors of soil formation we are able to state in advance what the soil must be like." This was one of the theses in the summary of Dokuchaev's doctor's dissertation.

One of Dokuchaev's collaborators, disciples, and followers was Sibirtzev. Indeed, some Russian soil investigators designate the Russian school of soil science as that of Dokuchaev and Sibirtzev. These two are considered as the founders and creators of the new school.

According to Sibirtzev (57, 59): "Under the term 'soil' we agreed to include what is known as the surface horizons of the parent material, in which the general dynamic processes are related to the biological processes. The variation in soils is determined: (a) by the parent material, i.e., its physico-chemical properties and position in space; (b) by the organisms, i.e., their kind, number, activity, and chemical transformations, resulting from it; and (c) by the physico-geographical conditions prevailing in the region during the process of soil formation and in their present final state." Sibirtzev considered moisture as the primary climatic factor in soil formation. In this his views coincide with those of Hilgard. He states [I am quoting from Glinka (19)]: "More important than the temperature is the humidity of the climate. Elsewhere enough was said about the primary and manifold influence of moisture on mechanical as well as chemical weathering. It is quite clear that in any isothermic belt the weathering of rocks varies (qualitatively and quantitatively) with the moisture conditions." In speaking of the climatic conditions in North America he stated: "The humidity conditions of the American climate change in an entirely different direction from those of European Russia: the loss in moisture does not follow the northwest-southeast direction, as in the southern half of European Russia, but the east and west. The eastern states are humid; the precipitation is twice as high as in our southern provinces. The western states, on the other hand, are very dry and are known among the Americans by the very inappropriate name 'arid region.' Correspondingly goes the distribution of soils."

It will be of interest to quote at this point the views of Sibirtzev (58) as to why the new concept of soils did not develop in the west [I am quoting from Afanasiev (2)]: "The causes which impeded the independent scientific study of soils in the west, and prevented the establishment of a genuine genetic classification of natural soils, were local, more or less accidental, due to external conditions, and were by no means of an essential nature.

"West-European scientists were less fortunate in this, for in most cases

they had to deal either with feebly developed soils, mixed with various geological deposits of inconsiderable thickness, or with eroded soils; and besides the soils have appreciably changed through cultivation.

"The methods of intensive and deep cultivation of the soils in the west, leaving out of consideration the introduction of various fertilizers, make them an artificially loosened mixture of natural soil material and of the underlying parent rock. The characteristic morphological horizons of the natural soil are either no longer or hardly distinguishable. The color and structure of the soil are altered and its composition tends to approach the composition of the parent material. Hence—the wide distribution of the geologico-petrographical and physico-chemical ideas on soil classification among the European scientists."

One of the prominent pupils of Dokuchaev was the late Dr. Glinka, whose volume on the distribution of soils—after having been translated into German—had a profound influence on the penetration of Dokuchaev's views into Germany and the United States.²

Glinka (19, 20), more than any of his predecessors, stressed the climate as a factor in the process of soil formation. He recognized, however, that in a number of cases the climatic factor may not be the predominating one and hence his divisions of *endodynamomorphic soils*, in which "the influence of the internal factors of soil formation (the properties of the parent material) definitely appears" and *ectodynamomorphic* in which climate as a factor in the process of soil formation is predominating.

We shall go no further in the historical development of Dokuchaev's ideas. Very new ideas developed on the concept of soil. The investigations of his followers deal to a great extent with soils as a natural body from the standpoint of soil classification. In this respect there is a wealth of material in the work of Nabokikh (42, 43), Visotzkii (70), Tumin (67), Kossovich (27, 28), Sabanin (53), Neustruev (44), Dima (9), Vilenski (69), Kostichev (29), Gedroiz (18), and a great number of others. In this paper we are not directly interested in this phase of the work and leave it for an opportune moment. A summary of the classification schemes as an outgrowth of Dokuchaev's views may be found in the paper of Afanasiev (2) and in the volume of Glinka (19).

The review of the development of the concept "soil" in historical perspective would be incomplete without the mention of some of the other German satellites, like Liebig and Ramann. Liebig (31, 32), whose influence for a while overshadowed all other currents in soil science, fundamentally paid but little attention to soils as such. For him the soil was the test tube in which one may introduce the chemical ingredients necessary for plant growth. The chemical composition of the plant was the criterion by which he judged soils. In his famous Letters (32, p. 122) Liebig quotes the definition of soils given by

² Dr. Marbut, Bureau of Soils, U. S. Department of Agriculture, translated Glinka's volume: "The great soil groups of the world and their development," and it is obtainable in mimeographed form.

Gustav Walz (1857), director of the Agricultural Academy at Hohenheim, Stuttgart: "The soil consists of disintegrated rocks, and either rests upon these same rocks or on others elsewhere; the transported soil may, nevertheless, have remained the same and corresponds at least to the rocks from which it has its origin." Liebig the chemist, the exponent of the classical "mineral theory," considered soils as the storehouse of the chemical components supplied by the minerals found in the disintegration products of rocks.

Ramann (48, 49), however, had a definite outlook on the genesis of soils and in a way his ideas were similar to those of Hilgard, inasmuch as he also laid down climate as the important factor in the process of soil formation and divided soils according to degrees of humidity under which they exist. Ramann (48) states: "It is my wish that my paper on the problem, which the work of the modern Russian scientists has advanced still further, should be published first in your country, where soil science has attained so vast and independent a development. . . . The problem related to the origin of certain soil types due to the effect of climatic conditions, has been first studied by Russian scientists, and among them the names of Dokuchaev and Sibirtzev will forever be connected with the development of this branch of science."

Modern definition of soils

A definite step forward in the definition of soils has been made by Marbut (37), the prominent American representative of the Dokuchaev school. His definition is as follows: "*The soil consists of the outer layer of the earth's crust usually unconsolidated ranging in thickness from a mere film to a maximum of somewhat more than ten feet which differs from the material beneath it, also usually unconsolidated, in color, structure, texture, physical constitution, chemical composition, biological characteristics, probably chemical processes, in reaction and morphology.*"

The definition purports to convey the idea about soils in terms of soil characteristics instead of soil forming processes as defined by the great majority of the followers of the Dokuchaev school.

Some of the later Russian investigators had the same viewpoint as Marbut, and Kossovich [Kossowitsch (27)] one of the most prominent among them says: "The sum-total of the physico-chemical and biological processes which act directly in the soil and manifest themselves in various forms is the natural basis for grouping soils. . . . The construction of a soil classification on the basis of coördinating individual factors of soil formation (parent material, climate, vegetation, position of the soil, age, etc.) as such was carried out by Sibirtzev. This was a great step forward. *However, the classification of soils with any one factor alone as the basis does not seem to be promising. The genetic soil classification should be based on the internal properties and characteristics of the soil itself.*"

No definition of soils based on the internal characteristics of the soil is offered by Kossovich in this paper, but in his book (28) he does define soils:

"All those surface horizons of the hard parent materials in which physico-chemical processes take place under the influence of the atmospheric agencies and in the presence of vegetation and animals." It may readily be seen that this definition is not as comprehensive as that of Marbut.

It seems to the author of this paper that any definition which attempts to convey the idea of soils as "independent, natural body," which in turn places the science of soils on the same level as the other natural sciences, should embody this statement. There is another point in connection with the definition of Marbut which one may take exception to, and that is the embodiment of the geologic concept "the outer layer of the earth's crust." It is not the "geologic concept part" that one may object to, but the sense of the phrase designating as soil the "outer layer of the earth's crust." The term "outer layer" may be synonymous with the term "surface layer" and our knowledge of soils as a natural body tells us that we may have soils not only in close relation to the surface but even below the surface. We have reference here to the buried soils studied by Visotzkii (70), Nabokikh (43), Florov (17) and others. These soils preserved their distinguishing characteristics and are distinct and well-defined soils when analyzed from the viewpoint of soils as a natural, historical body. A study of such soils may reveal the conditions under which the overlying soil formed. These soils may be studied as are other natural bodies buried in the earth's strata, such as fossils which gave rise to the science of paleontology. Similar to paleontology in geology we may have a branch of soil science which should deal with buried soils and *name this branch as paleopedology or paleoedaphology*, if the term edaphology is to be substituted for pedology as suggested by Shaw (55). Paleopedological studies of so-called fossil soils may reveal a lot of interesting geological data pertaining to climate.

Brevity of any definition is a desirable feature and it seems to the author that in Marbut's definition the clause: "ranging in thickness from a mere film to a maximum of somewhat more than 10 feet" may be omitted and instead the phrase "variable depth" substituted. As it stands there is an element of arbitrariness, which is not at all suggestive of the concept "soil."

The designations: "color, structure, and texture" may be omitted, since these are nothing more than some of the many other physical and morphological characters of soils. The words "probably chemical processes, in reaction" may also be omitted. The fact that the definition states the "chemical composition" (it should also include "properties") of the soil differs from that of the parent material implies a difference in chemical process. It is also clear that chemical properties of soils include, if anything, the reaction and the words "in reaction" are therefore not essential. With these explanatory remarks the definition of soils in terms of soil characteristics as suggested by Marbut (37) may be as follows: *The soil is a natural, historical body, of mineral and organic constituents, usually unconsolidated, of variable depth, which differs from the body of parent material below, also usually unconsolidated, in morphology, physical properties and constitution, chemical properties and composition, and*

biological characteristics. The introduction of the phrase "of mineral and organic constituents" in the definition seems to be justified on the ground that the combination of these constituents is the outstanding characteristic component of any soil.

The author is aware that there may be some loopholes in the modifications of Marbut's definition and the definition is presented here at this time with the hope that it might stimulate some other comments and result finally in a comprehensive logical and scientific definition of soils.

The discussion of the definition of the term "soil" would be incomplete without the mention of the one suggested by Shaw (55) in his comprehensive glossary of terms used in soil literature. It reads as follows: "The soil is a natural body occupying the surface portion of the earth, composed of mineral and organic materials and having more or less definitely developed horizons of eluviation and illuvation." The definition is accompanied by an explanation: "This term 'soil,' as defined, includes both the solum and the upper portion of the parent material, the A, B, and C horizons." The explanation as a support to the definition indicates the incompleteness of the definition, which in itself should be inclusive.

The definition as it stands does not convey the sum-total of soil characteristics. It is based on the characteristics of two horizons: eluvial³ and illuvial. And how about the "gley," the zone of effervescence? Why then include just two characteristics to the exclusion of others?

It has been pointed out that the fact of the usual location of the soil on the surface of the earth does not define soils; it is not a soil characteristic. An oak on the surface of the ground or buried in some geologic strata is an oak just the same. A soil, if buried, as long as it retains its soil characteristics is a soil just the same. Afanasiev (2) one of the leading Russian soil geographers and taxonomists considers the great service of Dokuchaev's views as consisting "in excluding soils from the system of surface cover formations and placing them into a distinct independent system of natural science (see p. 45)."

Morphology of soils

With the development of the scientific appreciation of soils, the methods of studying them have undergone radical changes, have been perfected and broadened. In the early history of soil science the viewpoint prevailing at the time determined the method of studying soils. Thus during the period of the geologic view the petrographic and mineralogical make-up of the soil was of primary importance. The agronomic point of view sought the mysteries of chemical reactions in the soil. Neither one of the soil science schools took

³ The term "eluvial" in connection with the A horizon, as the horizon of eluviation "from which material has been removed," is not altogether satisfactory. We must remember that hand in hand with the process of removal there is a process of accumulation also in the A horizon: the mineralization of the organic matter and humus accumulation.

up the systematic study of soils as they are and for this reason the logical approach to the study of any object; namely, its appearance, features, and general characters, in short the morphology of soils, had to wait until the science of the soil had been recognized as an independent science.

It is, therefore, natural that this phase of soil science should have developed first of all in Russia.

The first one to apply the morphological method in the study of soils, according to Zakharov (76), was Ruprecht (52), but the method has been developed by Dokuchaev and his pupils. Those who are interested in the historical aspect of the development of soil morphology may find an excellent review in the English paper of Zakharov (75), probably the most prominent morphologist among the Russian soil scientists living.

It was the new concept of soils as an independent, natural, historical body which required not only the description of the surface features of soil but also the anatomy of it; for this it is necessary to cut a vertical section and thus obtain a profile view of the exposed vertically dissected body. In this manner the morphology of soils is being studied.

From a morphological point of view the soil is a body definitely organized with a definite mode of construction, or build. It consists of a series of genetically related horizons formed from the parent material, with the aid of organic residues. As expressed by Tumin (67): "a soil may be looked upon as a body with a genetic complex of horizons formed in the process of humification and humus fixation." The morphological type of the soil imparts certain specific characteristics to the construction and constitution of the horizons; each type, so to speak, has a constant orderly system of relationships within the profile between the horizons. Thus in the zone of podzol soils there is a definite type of soil construction; the profile features are: under the dark leaf-mold layer we find a light gray horizon known as A_1 , followed by a lighter gray horizon A_2 , under which we find a darker horizon B, into which substances from the upper horizons are washed (mechanically and chemically), and under this horizon the parent material designated as C, is located.

Within each zone⁴ of soil formation the particular morphological type may develop on various kinds of parent material; we may therefore have podzols (a morphological term) on loess, on loam, on sands, etc. (mechanical and chemical composition and properties). And even within each morphologic type on a particular homogeneous parent material there may be subdivisions which manifest themselves in the soil construction. We may have at the border line

⁴ The division of soils into zones was original with Sibirtzev (57). It is based on the soil formation processes within a geographical region. In Russia these zones run parallel with the climatic belts. Thus the Russian workers separate European Russia and Siberia into: Tundra zone in the north; in the northern part of the temperate belt there is the podzol zone, followed by the forest steppe zone, then the chernozem zone, chestnut, and the gray-arid desert zones. The zonal divisions have been investigated by other workers, and the work of Afanasiev (1) is the outstanding contribution on the subject.

of the zonal belts intrazonal groups, in which the podzolization, for example, may not be well developed. This gives rise to a class of podzolized (not true podzols) soils. The leached grayish white horizon so characteristic for podzols is not very pronounced in podzolized soils. The general features of the podzolized group are, however, true to the morphological type of podzol soils.

The difference in the make-up of the horizons is the feature which determines the class or group in the morphological type.

According to Zakharov (75) the construction or make-up of a soil profile gives us three or four distinct genetic horizons: (a) The decomposition-organic accumulation; (b) eluvial; (c) illuvial, and (d) parent material immediately below the illuvial horizon. These horizons are indicated by the first letters of the alphabet, but there is no uniformity in assigning any one particular letter to the respective horizons. Thus Zakharov designates the horizons by A, B, C, and D. Other Russian investigators consider as A any horizon or subhorizon from which material is being washed down mechanically or chemically; B as the horizon of accumulation, compaction, and deposition; and C as the parent material. This latter designation seems to have become popular also among the few investigators on the continent and in the United States.

Stremme (65), one of the prominent representatives of the Dokuchaev School in Germany, credits Orth (47) as having been among the first to study the soil profile. By stretching the point one may agree with Stremme, but the facts of the case are that Orth studied the surface and subsurface of soils and subsoils. He noted the differences in the layers and pointed them out, but this does not mean a study of the profile in the genetic relationships. Such studies as those of Orth were made by many other early soil investigators of the geologic and agronomic school in Europe and the United States.

In recent years the Russian genetic school of soil science has been adopted by a great number of soil workers. The international soil conferences held in Budapest (1909), Stockholm (1910), Prague (1922), Rome (1924), and finally the First International Congress of Soil Science held in Washington (1927) firmly established the validity of the profile studies.

In the United States and Canada some studies on the soil profile have been made, but the outstanding contribution in this field has been made by Marbut (33, 34, 35, 36, 37, 38). In 1921 (34) the first survey was made in which the profile of the soil was described; and in making the report of the survey Marbut (34) justly states, "This report marks a definite step forward in soil study." It was the pioneer work of Marbut that established the study of the profile in the United States and Canada.

Joel (23) discusses the soil profile as a basis for classification and applies the soil profile idea to the soils of Canada. With climate as the basis of major grouping he divides the soils into sub-humid-arid and humid, pointing out the differences in profile characteristics. Fundamentally this mode of division is similar to that expressed by Hilgard, as previously discussed. There is, however, the changing viewpoint with respect to the appreciation of genetic

horizons. This viewpoint is an incomplete simile of the zonal idea developed by Sibirtzev and Afanasiev as shown before.

The profile studies of peat by Dachnowski (6, 7) may be mentioned, but these offer little for the orientation in the profile structure of soils. It is questionable whether one should apply the term horizon, as viewed by the genetic school of soil science, to peat layers. Peats in most cases are geological deposits, and one may speak of peat deposits but not of soil deposits. Peat layers are not a result of an internal arrangement due to a definite type of soil forming process.

A profile study in the podzol soil zone was made by Wheeting (73). A number of interesting points were brought out by the analyses of some of the physical and chemical properties of the horizons in the profile.

McCool, Veatch, and Spurway (39) report some physical and chemical studies on the profiles of some Michigan soils; McCool and Weidemann (40, 41) report further studies on soil profiles. The title of one of these papers (40) is misleading and really does not in any way touch upon the subject of the soil profile. A study of the profile of forest soil in relation to reaction has been reported by Spokes (61). From this paper it is not clear how the horizons in the profile have been determined. The distribution of nitrogen in the profile of podzol soils has been studied by Edington and Adams (15). Other recent papers dealing with certain phases of the subject are those of Lebedev (30), Norton and Smith (46), and Spurway and Austin (63). Holmes (24a) studied the colloidal properties of several profiles on a silt loam soil.

A series of papers on soil profile studies appeared in the Proceedings and Papers of the First International Congress of Soil Science. Of these the paper by Joel (24) is of interest. It takes up the profile study within the several zones of soil formation; the few chemical analyses presented illustrate the validity of the field studies when made from the morphological standpoint. The micro-relief and origin of parent material introduce within any one zone patches of soil which should belong to a different zone. This has been pointed out by Tumin (67), Kossovich (27), and others.

A very instructive paper is the one by Baldwin (3) on the gray-brown podzolic soils of the eastern United States. A description of some soil profiles in Illinois is given by Norton (45). He attempts to establish a correlation between topography and drainage on the one hand and some soil characters on the other. Shaw (56) sketches the profile development in the secondary (immature) soils of California. Smolik (60) describes and gives the analyses of podzol soils in Czechoslovakia with special reference to the composition and behavior of the eluvial and illuvial horizons. Dachnowski-Stokes (8) makes an interesting comparison between peat profiles and peat soils. He rightly designates as layers or strata (a geologic concept) the profile constitution of peat; whenever the peat has been worked over by the forces of soil forming processes, become humified and mineralized as a result of which an

organic soil is formed with peat as the parent geologic material, he uses the term horizon in designating the profile constitution.

A valuable contribution to the study of the podzols is the paper by Veatch (68); it gives a clear picture of the soil forming processes in the region described. The paper of Wyatt and Newton (74) would have been more valuable if the chemical data included the aluminum and iron content of the soils described and illustrated.

The profile of the unique Cuban soils are lucidly described by Bennett (4). A reaction study of the soil profile in some Oregon soils is presented by Stephenson (64). The profile study of the microbial flora in Iowa, by Brown and Benton (5a) should be mentioned.

A large number of papers on the subject of the soil profile appeared in Russia. A review of only those which have appeared in recent years would necessitate a separate paper. The originals of these papers are in a large number of cases not accessible and besides most of these papers deal with profile studies of the various soil zones from the standpoint of soil classification. As the latter point is not the object of this review, it was deemed advisable to leave the papers out for a more opportune occasion.

The study of the soil profile is at present an indispensable part in any branch of soil science. The soil morphologist, the soil surveyor, the physicist, the chemist, the microbiologist, even the agronomist, all have turned their attention to the soil profile, its constitution and its make-up in horizons, for the study of soils as a natural, historical body is possible only upon exposing the body in its cross-section, which is the same as the profile.

The methods used in the study of the profile of some New Jersey soils and some of the results will be the subject of a forthcoming paper.

SUMMARY

1. A discussion is presented on the development of soil science as an independent science.
2. The ideas on soil and the various schools of soil genesis are reviewed with special reference to the Russian school from the time of Dokuchaev to date.
3. The definitions of soil as given by Marbut and Shaw are critically analyzed and a modification of the Marbut definition is presented.
4. The suggestion is made to consider a branch of soil science to be known as paleopedology or paleoedaphology for the study of buried soil.
5. The soil morphology and the soil profile are discussed and a review of the subjects is presented.

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COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: IV. THE NATURE AND RAPIDITY OF DECOMPOSITION OF THE VARIOUS ORGANIC COMPLEXES IN DIFFERENT PLANT MATERIALS, UNDER AEROBIC CONDITIONS

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The rapidity of decomposition of different organic substances of either plant or animal origin in soil is controlled largely by four distinct factors:

(a) The chemical composition of the organic material, which, in the case of plants and plant remains, depends primarily upon the nature and age of the plant as well as upon the conditions of its nutrition. (b) The presence of sufficient nitrogen to enable the microorganisms bringing about the decomposition to carry out this process in the shortest possible time; in the case of nitrogen-rich plant residues, as in young plants used for green manuring purposes, or legume residues, this never becomes a limiting factor; however, in the case of plant residues poor in nitrogen, such as straw, the problem may become one of considerable importance. (c) The nature of microorganisms active in the decomposition processes. (d) The environmental conditions at which decomposition is carried out, especially aeration, moisture supply, soil reaction, and temperature.

To throw light upon these important phases of the problem of decomposition of organic residues in the soil, the following experiments were undertaken. Four different plant materials, varying distinctly in chemical composition, were selected for this purpose; namely, (a) mature corn stalks (and leaves); (b) rye straw; (c) mature, yellow oak leaves, freshly fallen to the ground; (d) mature alfalfa plants, freshly harvested.

Each of these plant materials was collected in sufficient quantity and passed separately through a chopper, which cut them into small pieces. After the moisture content of the plant material was determined, a sufficient quantity of each was added to a series of glazed earthenware pots, to give 200 to 280 gm. of dry material in each pot. Sufficient water was then added to each pot to bring the total moisture content of the compost to 66.6 per cent, or to 200 per cent moisture on the basis of the dry organic matter.

Half of the pots received a mineral solution containing 1 gm. $(\text{NH}_4)_2\text{HPO}_4$, 1 gm. K_2HPO_4 , and 2 gm. CaCO_3 . The nutrients thus added were far from sufficient to allow a very rapid decomposition of the nitrogen poor plant materials, such as the rye straw; at least five times as much additional nitrogen would have been required by the microorganisms for the complete decomposition of the celluloses and hemicelluloses in this plant material. All the pots

were inoculated with a suspension of fresh soil, covered with plates, and incubated at 25 to 28°C.

At intervals, the residual organic matter in each pot was weighed, the moisture content determined, and an aliquot portion taken for analysis. The results were always calculated on the basis of both the percentage of the residual material and the total original material, making thereby due allowance for the samples removed at different times for chemical analysis; the last calculation would enable one to determine just how much of the total amount of each of the different plant constituents had been decomposed in the given period of time.

Various methods have been used in the past for following the course of decomposition by microorganisms of plant material or animal residues, either in soil or in compost. These methods were usually based upon measuring only one product of decomposition, such as ammonia and frequently nitrate or carbon dioxide. The assumption was thereby made that under a definite set of conditions, the liberation of these various products of microbial metabolism runs parallel to the total decomposition of the organic residues as a whole as well as of their various constituents. This assumption is not always justified. In the case of a plant substance containing only a small amount of nitrogen, very little free ammonia or nitrate will be produced even after considerable decomposition has taken place. This is because most of the inorganic nitrogen, which becomes liberated as a result of the decomposition of the organic nitrogenous constituents of the plant substance, is reassimilated by the microorganisms; the latter utilize the energy liberated in the process of decomposition of the celluloses and hemicelluloses. Evolution of carbon dioxide is a more direct index of decomposition, although the disintegration of the same amount of cellulose will yield varying amounts of carbon dioxide when carried out by different organisms and under different conditions. Further, this method tells nothing at all as to the particular chemical complexes among the plant constituents which are undergoing decomposition. To speak of the decomposition of the plant material as a whole, as measured by the evolution of carbon dioxide, is to neglect the nature of the numerous chemical processes which are taking place thereby. Another important limitation of these methods for studying decomposition of organic matter is that little information is gained concerning the chemical nature of the residual material which results from decomposition and which goes to increase the "humus" content of the soil.

Attempts have been made in some instances to measure the decomposition of plant material either by determining the total reduction in the bulk of the organic matter or by measuring the disappearance of one of the important constituents. However, the mere fact that 40, 60, or 80 per cent of the organic matter of the specific plant material has disappeared, under certain conditions of decomposition and in a definite period of time, supplies no in-

formation whatsoever concerning the chemical changes that have taken place in the various constituents.

Studies dealing with the disappearance of specific chemical complexes in the organic material undergoing decomposition have been limited largely to the celluloses and hemicelluloses. Barthel and Bengtsson (2) have shown that the decomposition of celluloses in plant residues depends upon the nature of the plant and upon the amount of available nitrogen. Cellulose in the stubble and roots of legumes decomposed more slowly than did cellulose in the roots of cereal straws. This was explained by the greater content of nitrogen-free, non-cellulosic, carbonaceous materials in the former than in the latter. Fraps (5) observed that, as a result of decomposition of different plant materials in soil for a period of eight weeks, there were left 7 per cent of the pentosan in cotton seed meal, 31 per cent of the pentosan in Sudan grass, 61 per cent of the pentosan in rice bran, and 75 per cent of the pentosan in sheep manure. These results point definitely to differences in the decomposition of the same group of organic complexes in different plant materials.

Schmidt, Peterson, and Fred (11) found that when corn fodder and rye straw were undergoing decomposition under the same conditions, the former lost 50 per cent of its pentosans in 100 days and the latter only 35 per cent in 300 days; they also observed that common fungi synthesize pentosans, even in media free from pentose material. According to Rege (9), 80 per cent of the pentosan in fresh rye straw, when the straw is allowed to decompose under favorable conditions, disappeared in 40 days; in fact during the early period of decomposition, that is between the fourth and eighth days, practically all the loss in dry matter was accounted for almost entirely by the loss of pentosan.

Very few attempts have been made to measure the rate of disappearance of the various chemical complexes in the decomposing plant materials. The investigations of Egorov (6), Bach (1), and König (7) can be summarized as follows: During the early stages of decomposition of plant materials in soil or in compost, the pentosans disappear more rapidly than do the total organic constituents of the material, and even more rapidly than do the celluloses. After considerable decomposition has taken place, the celluloses are found to disappear more quickly than the hemicelluloses (including the pentosans). The rate of decomposition of both hemicelluloses and celluloses is greater than that of the total organic matter, as shown by the amount of residual material. Organic nitrogenous compounds tend to accumulate, especially in the case of nitrogen-poor organic residues, so that the total protein content of the residual undecomposed material diminishes only slowly when compared with the rate of disappearance of the total organic matter. Lignins seem to be resistant to decomposition, more so than any other group of the major plant constituents; they are found, therefore, to accumulate in the process of decomposition of plant residues, with certain few exceptions.

Bach (1) found that when stable manure is added to the soil and allowed to decompose, the per cent of carbon in the residual organic matter increases

from an initial 47 to 51 per cent to 58 per cent. This was explained by the fact that the celluloses and pentosans originally present in the fresh manure contained 44.44 and 45.44 per cent of carbon respectively, while the lignins and cutins contained 67.31 to 71.35 per cent of carbon. As a result of decomposition, the celluloses and pentosans disappear rapidly while the lignins and cutins accumulate, thus leading to a gradual increase in carbon content.

Rose and Lisse (10), analyzing wood at different stages of decomposition, and Bray and Andrews (3) working with pure cultures of fungi, also demonstrated that, in the decomposition of wood by various Hymenomycetes, the pentosans and celluloses are first to disappear, whereas the lignins are practically resistant to decomposition. Only very few fungi, namely those causing the "white rots," are capable of decomposing lignins as well as celluloses (4). The common wood-destroying fungus *Merulius lacrymans* decomposes the celluloses while the lignins are left; these are converted partly into depolymerized and split substances of a "humin-like" nature.

It has also been found that, as a result of the decomposition of plant substances there is a marked increase in the alkali-soluble material of the undecomposed or accumulated residues. The tendency has been to explain this phenomenon either by a modification of the lignin molecule, making it more readily soluble in alkali, or by the formation of some intermediary products of cellulose decomposition soluble in alkalies.

Waksman and Tenney (14) came to the conclusion, as a result of studies on the decomposition of the rye plant harvested at different stages of growth, that the water-soluble organic substances are first to be decomposed in the soil by microorganisms. This is soon followed by an attack upon the pentosans and at the same time, or immediately after, upon the celluloses. Although the plant residues contain a larger amount of celluloses than of hemicelluloses and although the latter begin to undergo decomposition sooner than the former, the celluloses disappear sooner and more completely than the hemicelluloses. This was explained by the fact that, whereas pentosans may be attacked more quickly and by a greater variety of microorganisms than celluloses, other hemicelluloses (galactans, mannans) may be more resistant; further, as a result of the growth of microorganisms upon the plant residues, considerable quantities of hemicelluloses are synthesized in the form of bacterial and fungus slimes and gums. The lignins were found to be more resistant to decomposition and tend to accumulate in the soil. The plant proteins are readily decomposed, but very little nitrogen becomes liberated in the soil as ammonia, as long as there is left a considerable amount of undecomposed cellulose and pentosan. Accompanying the decomposition of these carbohydrates, considerable synthesis of microbial proteins takes place. This synthesis is also a result of the growth of the microorganisms bringing about the decomposition of the organic matter.

The dark residues resulting from the decomposition of the plant material, or the so-called "humus" tends to have the following composition: a large

amount of lignins or modified lignins of plant origin; a large amount of protein of microbial origin; a fairly high hemicellulose content, partly of plant and partly of microbial origin; small amounts of plant constituents still undergoing decomposition, such as celluloses and ether-soluble substances; small amounts of other synthesized microbial products, some of which are undergoing decomposition, as various fatty substances, chitinous materials and nitrogenous complexes.

An attempt will be made in this paper to elucidate the problem of decomposition of different plant materials under aerobic conditions, to establish the relation between the different organic plant constituents in the process of

TABLE 1
Proximate composition of plant materials used for decomposition studies
On per cent basis of dry material

| CHEMICAL CONSTITUENTS | CORN STALKS | RYE STRAW | OAK LEAVES | ALFALFA PLANTS |
|------------------------------|----------------|--------------|---------------|-------------------|
| Ether-soluble fraction | 1.80 | 1.84 | 3.71 | 2.75 |
| Cold-water-soluble | 10.58 | 4.51 | 8.28 | 12.44 |
| (Reducing sugar) | (6.00) | (0.88) | (2.73) | (1.31) |
| Hot-water-soluble | 3.56 | 1.75 | 5.65 | 4.80 |
| (Alcohol-soluble*) | (4.19) | (3.49) | (5.92) | (7.66) |
| Hemicelluloses | 17.63 | 21.10 | 12.93 | 8.52 |
| Celluloses | 29.67 | 38.62 | 13.78 | 26.71 |
| Lignins | 11.28 | 14.63 | 30.30 | 10.78 |
| Crude protein | 1.98 | 0.81 | 4.25 | 8.13 |
| (Total nitrogen) | (0.66) | (0.24) | (0.77) | (2.58) |
| Ash | 7.53 | 4.18 | 5.09 | 10.30 |

* The alcohol-soluble fraction was determined on a separate sample. In view of the fact that this fraction was not determined in the decomposed material, it is left out of further consideration; it is given here, however, merely for the purpose of comparison.

decomposition, and to throw further light upon the rôle of microorganisms in the processes of decomposition and synthesis.

It is quite essential, in a study of this nature, to be able to make as complete an analysis of the organic plant constituents as possible. Such an analysis has been proposed elsewhere (12) and was used to considerable advantage for determining the proximate composition of various plant materials. It was found that, by this method of analysis, 85 to 96 per cent of the plant constituents (except in the case of mosses) could be accounted for. No attempt has been made to measure those complexes which occur in the plant in concentrations of less than one per cent.

The following organic complexes are accounted for in this method:

(a) cold-water-soluble substances, including the sugars and amino acids; (b) hot-water-soluble substances, including starches, pectins, tannins, and uric acid; (c) hemicelluloses, determined by hydrolysis with hot dilute mineral acid and measured in the form of reducing

sugars; (d) celluloses, determined by hydrolysis with cold 80 per cent sulfuric acid followed by boiling for several hours, after diluting with 15 volumes of water; (e) lignins, as determined by their insolubility in cold 80 per cent sulfuric acid, with ash and nitrogen accounted for; (f) ether-soluble substances, including fats and waxes; (g) crude proteins.

In view of the fact that the alcohol-soluble fraction is not included in the following analyses, the results will be short 3.5 to 7.5 per cent of the plant constituents. However, since the same method has been used in the analysis of both the fresh material and the decomposed residues, the results are found to be comparable. The proximate chemical composition of the four materials used in the following investigations is given in table 1. The corn stalks were harvested when mature but not fully dry, some of the leaves being still green. The rye straw was in a fully mature stage, as shown by the low nitrogen

TABLE 2

Chemical composition of corn stalks and decomposed residue at different stages of decomposition, without the addition of nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | | |
|-------------------------|-------------------------------|-----------------------------|-------|-------|-------|
| | | 27 | 68 | 205 | 405 |
| Ether-soluble..... | 1.80 | 1.97 | 0.96 | 0.78 | 0.35 |
| Cold-water-soluble..... | 10.58 | 3.37 | 5.74 | 2.80 | 4.28 |
| Hot-water-soluble..... | 3.56 | 2.50 | 3.02 | 4.11 | 9.19 |
| Hemicelluloses..... | 17.63 | 16.34 | 15.93 | 15.35 | 10.74 |
| Celluloses..... | 29.67 | 26.36 | 22.50 | 13.39 | 4.78 |
| Lignins..... | 11.28 | 18.26 | 19.66 | 23.44 | 23.86 |
| Crude protein..... | 1.98 | 4.37 | 4.69 | 9.00 | 12.97 |
| Ash..... | 7.53 | | | 19.16 | 26.63 |

content. The oak leaves were also mature and recently fallen; they were collected from the surface of ground under the trees. The alfalfa plants were freshly harvested in the fall of the year.

Attention should be called to the fact that the crude protein figures indicate the total nitrogen minus the water-soluble nitrogen, the difference being multiplied by 6.25. The total nitrogen figures are given separately.

DECOMPOSITION OF CORN STALKS

The corn stalks contained 66 per cent moisture when harvested, showing that they were not as yet fully mature. The material was air-dried and subjected to a detailed chemical analysis, the results of which are given in table 1. For the study of aerobic decomposition, the fresh material was cut up immediately and used without preliminary drying. In view of the fact that the moisture content of the fresh material was just sufficient for optimum aerobic decomposition, no more water was added. The analysis of the dry material shows that it contained 10.58 per cent of organic substances soluble

in cold water. More than half of this, namely 6 per cent, was in the form of reducing sugars. This high concentration of sugar explains the rapid initial decomposition of the corn stalks. Out of the 0.66 per cent total nitrogen in the dry material, 0.26 per cent, or nearly 40 per cent of the total nitrogen,

TABLE 3

Chemical composition of corn stalks and the decomposed residue at different stages of decomposition, in the presence of added nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | | |
|-------------------------|-------------------------|-----------------------------|-------|-------|-------|
| | | 27 | 68 | 205 | 405 |
| Ether-soluble..... | 1.80 | 2.22 | 0.80 | 0.64 | 0.25 |
| Cold-water-soluble..... | 10.58 | 3.43 | 5.27 | 3.96 | 4.59 |
| Hot-water-soluble..... | 3.56 | 2.45 | 3.20 | 5.36 | 8.71 |
| Hemicelluloses..... | 17.63 | 15.56 | 16.41 | 10.68 | 10.39 |
| Celluloses..... | 29.67 | 23.80 | 21.93 | 6.28 | 5.05 |
| Lignins..... | 11.28 | 17.70 | 19.12 | 23.83 | 21.30 |
| Crude protein..... | 1.98 | 4.81 | 6.84 | 10.93 | 12.13 |
| Ash..... | 7.53 | | | 26.12 | 29.43 |

TABLE 4

Total decomposition of the various organic chemical constituents of corn stalks, without the addition of nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | MATERIAL LEFT AFTER DAYS OF DECOMPOSITION | | | | | | | |
|--|-------------------|---|----------------------|---------------|----------------------|---------------|----------------------|---------------|----------------------|
| | | 27 | | 68 | | 205 | | 405 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | | gm. | |
| Total dry material..... | 203.00 | 129.00 | 63.54 | 92.50 | 45.60 | 59.50 | 29.30 | 41.20 | 20.30 |
| Ether-soluble fraction.... | 3.65 | 2.54 | 69.59 | 0.89 | 24.38 | 0.46 | 12.60 | 0.14 | 3.84 |
| Cold-water-soluble organic matter..... | 21.48 | 4.35 | 20.25 | 5.31 | 24.72 | 1.67 | 7.77 | 1.76 | 8.19 |
| Hot-water-soluble organic matter..... | 7.23 | 3.23 | 44.67 | 2.79 | 38.59 | 2.45 | 33.89 | 3.79 | 52.42 |
| Hemicelluloses..... | 35.79 | 21.09 | 58.93 | 14.74 | 41.18 | 9.13 | 25.51 | 4.42 | 12.35 |
| Celluloses..... | 60.24 | 34.00 | 56.44 | 20.81 | 34.55 | 7.97 | 13.23 | 1.97 | 3.27 |
| Lignins..... | 22.90 | 23.56 | 102.88 | 18.19 | 79.43 | 13.95 | 60.92 | 9.83 | 42.93 |
| Crude protein..... | 4.01 | 5.64 | 140.65 | 4.34 | 108.23 | 5.36 | 133.67 | 5.34 | 133.17 |

was in a water-soluble form. These facts, as well as the low lignin content of the corn stalks, account for the rapid disappearance of most of the organic constituents in their decomposition, especially in the presence of a small amount of inorganic salts of nitrogen, phosphorus, and potassium.

Tables 2 and 3 give the chemical composition of the corn stalks, with and

without the additional nutrients, at different stages of decomposition. Tables 4 and 5 show the amount of total decomposition that the various organic chemical constituents have undergone, in the absence and in the presence of added nutrient salts, as shown by the concentrations of the various groups left at the different periods of incubation. These figures were obtained by multiplying the total residual material (on a dry basis and after allowance has been made for the samples removed) by the percentage composition.

Figures 1 and 2 illustrate the rapidity of transformation of the most important groups of organic plant complexes, in the process of decomposition of corn stalks, with and without additional nutrient salts. The left hand columns represent the abundance of the particular complex in the original undecom-

TABLE 5

Total decomposition of the various organic chemical constituents of corn stalks in the presence of added nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | MATERIAL LEFT AFTER DAYS OF DECOMPOSITION | | | | | | | |
|--|-------------------|---|----------------------|---------------|----------------------|---------------|----------------------|---------------|----------------------|
| | | 27 | | 68 | | 205 | | 405 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gms. | gm. | | gms. | | gms. | | gms. | |
| Total dry material..... | 203.00 | 107.30 | 52.86 | 83.20 | 41.00 | 53.80 | 26.50 | 45.70 | 22.50 |
| Ether-soluble fraction.... | 3.65 | 2.38 | 65.21 | 0.67 | 18.36 | 0.34 | 9.32 | 0.11 | 3.01 |
| Cold-water-soluble organic matter..... | 21.48 | 3.67 | 17.09 | 4.38 | 20.39 | 2.13 | 9.92 | 2.10 | 9.78 |
| Hot-water-soluble organic matter..... | 7.23 | 2.62 | 36.24 | 2.66 | 36.79 | 2.88 | 39.83 | 3.98 | 55.05 |
| Hemicelluloses..... | 35.79 | 16.65 | 46.52 | 13.65 | 38.14 | 5.75 | 16.07 | 4.75 | 13.27 |
| Celluloses..... | 60.24 | 25.46 | 42.26 | 18.25 | 30.30 | 3.38 | 5.61 | 2.31 | 3.83 |
| Lignins..... | 22.90 | 18.94 | 82.71 | 15.91 | 69.48 | 12.82 | 55.98 | 9.73 | 42.49 |
| Crude protein..... | 4.01 | 5.16 | 128.68 | 5.69 | 141.90 | 5.88 | 146.63 | 5.54 | 138.15 |

posed plant material, on the basis of 100 per cent. The second column to the right of it represents the concentration, in per cent, of the particular complex at the time of the first sampling, namely after 27 days; the third, fourth, and fifth columns to the right represent the concentrations of the particular complex at the second, third, and fourth sampling; namely, after 68, 205, and 405 days, respectively.

Both the tables and figures give a fair idea of the processes which have been taking place in the decomposition of corn stalks under aerobic conditions. The addition of inorganic nutrients exerted a decidedly favorable effect upon the early stages of decomposition of the total organic matter, especially of the celluloses and hemicelluloses. After 27 days, 36.5 per cent of the total organic constituents of the corn stalks disappeared in the absence of the additional salts and 47.1 per cent in their presence; 41 per cent of the hemi-

celluloses and 43.6 per cent of the celluloses were decomposed in the absence of added nutrients, whereas, in their presence, the disappearance of these two groups of complexes was 53.5 and 57.7 per cent respectively. These results induce certain important conclusions: 1. The addition of available nitrogen was responsible for a greater decomposition of the celluloses and hemicelluloses in the corn stalks, especially during the early stages of decomposition. 2. Both the hemicelluloses and the celluloses disappeared more quickly than the total organic matter; this indicates either that synthesis of new complexes has taken

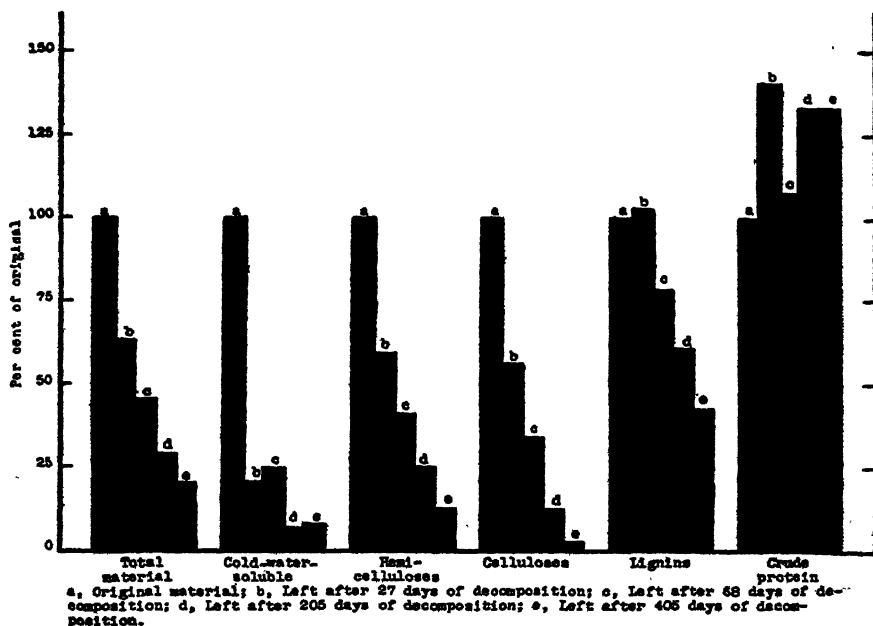


FIG. 1. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF CORN STALKS WITHOUT ADDITIONAL NUTRIENT SALTS

place or that some of the more resistant plant complexes have accumulated. Both assumptions are correct, as is shown later.

The favorable effect of additional inorganic nutrients upon decomposition is still marked after 68 days of decomposition, but this effect tends to disappear later. It is interesting to note also that at the beginning of decomposition the hemicelluloses decomposed as rapidly as if not more so than the celluloses; however, during the latter stages, the celluloses disappeared rapidly, while appreciable quantities of hemicellulose were still left in the residual compost.

The cold-water-soluble substances tend to disappear rapidly when decomposition sets in. The hot-water-soluble constituents decompose more slowly and even tend to accumulate during the later stages of decomposition, no

doubt because of the production of synthesized microbial cell substance containing substances soluble in hot water. The fats and oils, or the ether-soluble substances, of the corn stalks were also rapidly decomposed under aerobic conditions.

The lignins and the proteins are the two important complexes among the various organic plant constituents which tend to accumulate. The reason for their accumulation, however, is not the same in both cases. The lignins are more resistant to attack by microorganisms than are the other groups of plant constituents, such as the celluloses, hence they accumulate as the decomposition

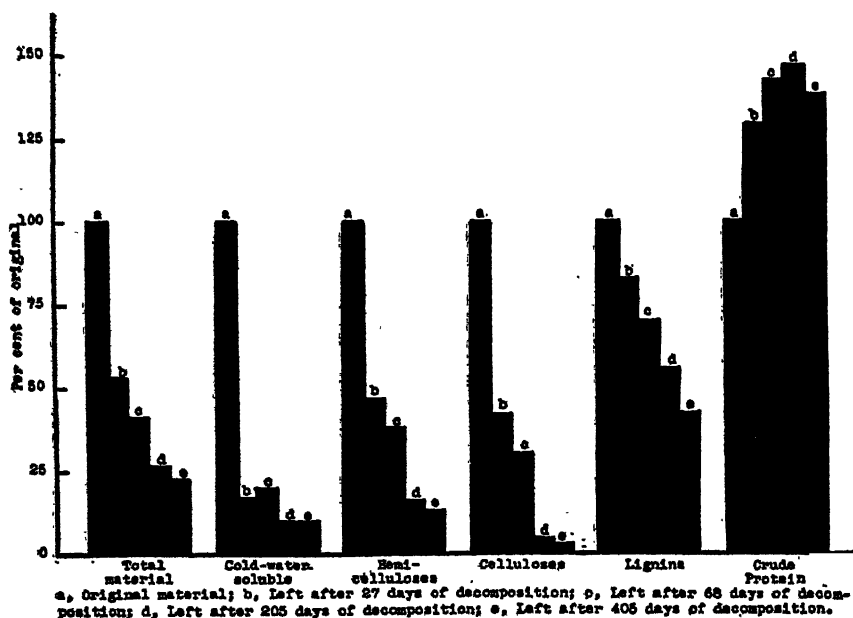


FIG. 2. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF CORN STALKS WITH ADDITIONAL NUTRIENT SALTS

of the more readily available complexes proceeds further. In 27 days, only a small fraction of the lignins of the corn stalks undergoing decomposition under aerobic conditions has disappeared; this accounts for the increasing concentration of the lignin content of the residual material. The original plant substance had only 11.28 per cent lignin; the material decomposed for 405 days contained twice as much lignin in proportion to the other constituents. However, there was no absolute preservation of the lignin complex, as assumed by some investigators. Under aerobic conditions, the lignins gradually decompose, not so rapidly by far as the other chemical complexes, but still in a very definite manner; after 405 days, 57.5 per cent of the lignin in the corn stalks has actually disappeared. Under anaerobic conditions, the

lignins are preserved to a much greater extent, as will be shown in a later contribution.

These results as well as those obtained on the decomposition of the other three plant materials prove definitely that lignins decompose under aerobic conditions. So far no definite explanation can be submitted concerning the nature of the microorganisms active in this process. It is known that certain *Basidiomycetes* are capable of decomposing lignins in the rotting of trees. It is also known that lignins disappear partly when digested by animals; the limited evidence points to actinomyces as possible agents in the decomposition of lignins. Whether one of these three groups of organisms was responsible for the disappearance of some of the lignins in the decomposition studies reported here or all three groups of organisms took a part in the process still remains to be established.

TABLE 6
Nitrogen transformation in the decomposition of corn stalks
In per cent of total residual material

| FORM OF NITROGEN | NO ADDITIONAL NITROGEN | | | | | AMMONIUM PHOSPHATE ADDED | | | | |
|---|------------------------|---------|---------|----------|----------|--------------------------|---------|---------|----------|----------|
| | Incubation | | | | | Incubation | | | | |
| | 0 days | 27 days | 68 days | 205 days | 405 days | 0 days | 27 days | 68 days | 205 days | 405 days |
| Total nitrogen..... | 0.66 | 0.95 | 1.02 | 1.70 | 2.44 | 0.76 | 1.05 | 1.37 | 2.10 | 2.34 |
| Soluble in cold water..... | 0.25 | 0.13 | 0.14 | 0.13 | 0.12 | 0.35 | 0.16 | 0.14 | 0.14 | 0.14 |
| Soluble in hot water..... | 0.09 | 0.12 | 0.13 | 0.13 | 0.25 | 0.09 | 0.13 | 0.14 | 0.21 | 0.26 |
| Hydrolizable by 2 per cent HCl..... | 0.13 | 0.23 | 0.36 | | 0.92 | 0.13 | 0.28 | 0.39 | | 0.93 |
| "Humin" nitrogen, not acted upon by autoclaving with 6 per cent H ₂ SO ₄ | 0.13 | 0.24 | 0.33 | 0.44 | 0.51 | 0.13 | 0.27 | 0.33 | 0.51 | 0.51 |

The increase in the crude protein (insoluble in cold and hot water) with the advance of decomposition of the corn stalks was not only relative to the other plant constituents, but there was an actual total increase in the amount of protein, due to the transformation of the water-soluble nitrogen compounds into insoluble complex organic nitrogenous substances. The greatest increase in the protein content was obtained in those preparations which received inorganic nitrogen salts. The reasons for the increase in the protein as a result of decomposition of nitrogen-poor organic plant residues have been expounded in detail elsewhere (15). These results throw further light upon the problem of synthesis of new protein material as a result of the activities of the microorganisms which bring about the decomposition of the celluloses and hemicelluloses. The fact that, in the absence of additional nitrogen, the increase in the amount of crude protein took place at the expense of the water-soluble simple nitrogenous compounds is brought out in table 6. Although the total nitrogen of the residual material rapidly increased with the advance

in decomposition, the water-soluble nitrogen decreased. The acid hydrolyzable nitrogen increased even more rapidly than the total nitrogen. It is interesting to record the marked increase in the so-called "humin" nitrogen or that part of the nitrogen which is found in the lignin fraction. These results point definitely to the building up of resistant nitrogenous complexes by the microorganisms.

As the water-soluble and insoluble nitrogenous compounds, first in the form of plant constituents then as constituents of microbial cells undergoing decomposition, are repeatedly hydrolyzed and resynthesized by microorganisms, more and more of the resistant nitrogenous complexes are produced. These are attacked less and less readily, and finally become incorporated in the residual material equivalent to the soil "humus" with its large content of organic nitrogenous complexes resistant to decomposition.

TABLE 7

Chemical composition of rye straw and its decomposition products at different stages of decomposition, without the addition of nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | |
|--------------------------|-------------------------|-----------------------------|-------|-------|
| | | 66 | 143 | 386 |
| Ether-soluble | 1.84 | 1.05 | 1.33 | 1.62 |
| Cold-water-soluble | 4.51 | 2.12 | 2.45 | 1.75 |
| Hot-water-soluble | 1.75 | 2.19 | 2.23 | 2.08 |
| Hemicelluloses | 21.10 | 19.81 | 21.25 | 18.18 |
| Celluloses | 38.52 | 35.33 | 33.47 | 30.38 |
| Lignins | 14.63 | 17.53 | 17.59 | 18.88 |
| Crude protein | 0.81 | 1.69 | 1.88 | 2.76 |
| Ash | 4.18 | 5.50 | 5.60 | 5.72 |

DECOMPOSITION OF RYE STRAW

The chemical composition of the rye straw used in these decomposition studies varied in several respects from that of the corn stalks. The straw had much less water-soluble material, much less nitrogen, but a larger proportion of celluloses, hemicelluloses, and lignins. One would expect from this analysis that the decomposition of the straw should proceed at a much slower rate than that of the corn stalks. This was actually found to be the case.

The straw, undergoing decomposition under the same conditions as the corn stalks, was analyzed only three times; namely, after 66, 143, and 386 days incubation at 25–28°C. Tables 7 and 8 give the chemical composition of the straw without and with the same additional inorganic salts at different stages of decomposition. Tables 9 and 10 and figures 3 and 4 give the total corresponding amounts of the different chemical complexes of the fresh and decomposed material at the different stages of decomposition.

In comparison with the corn stalks, the rye straw was found to decompose very slowly. Although the nature and course of decomposition of the straw are similar to that of the corn product, the amount decomposed is considerably less. At the end of 386 days, nearly three times as much organic matter was left from the straw as from the corn stalks. This is due largely

TABLE 8

Chemical composition of rye straw and its decomposition products at different stages of decomposition, in the presence of added nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | |
|--------------------------|-------------------------|-----------------------------|-------|-------|
| | | 66 | 143 | 386 |
| Ether-soluble | 1.84 | 0.92 | 1.14 | 0.59 |
| Cold-water-soluble | 4.51 | 2.05 | 2.21 | 1.76 |
| Hot-water-soluble | 1.75 | 1.89 | 1.81 | 1.80 |
| Hemicelluloses | 21.10 | 19.61 | 21.10 | 17.98 |
| Celluloses | 38.62 | 33.58 | 31.02 | 25.99 |
| Lignins | 14.63 | 18.10 | 18.28 | 18.43 |
| Crude protein | 0.81 | 2.25 | 2.38 | 3.53 |
| Ash | 4.18 | 6.50 | 6.50 | 8.00 |

TABLE 9

Total decomposition of the various organic constituents of rye straw, without the addition of nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC MATTER LEFT, AFTER DAYS OF DECOMPOSITION | | | | | |
|---|-------------------|--|----------------------|---------------|----------------------|---------------|----------------------|
| | | 66 | | 143 | | 386 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | |
| Total dry material | 277.00 | 230.00 | 83.03 | 197.00 | 71.12 | 171.00 | 61.73 |
| Ether-soluble fraction | 5.10 | 2.42 | 47.45 | 2.62 | 51.37 | 2.77 | 54.31 |
| Cold-water-soluble organic matter | 12.49 | 4.88 | 39.07 | 4.83 | 38.67 | 2.99 | 23.94 |
| Hot-water-soluble organic matter | 4.85 | 5.04 | 103.92 | 4.39 | 90.52 | 3.56 | 73.40 |
| Hemicelluloses | 58.42 | 45.56 | 77.99 | 41.86 | 71.65 | 31.09 | 53.22 |
| Celluloses | 106.66 | 81.25 | 76.18 | 65.94 | 61.82 | 51.95 | 48.71 |
| Lignins | 40.51 | 40.32 | 99.53 | 34.65 | 85.53 | 32.28 | 79.68 |
| Crude protein | 2.25 | 3.88 | 172.44 | 3.70 | 164.44 | 4.72 | 209.78 |

to the insufficient decomposition of the celluloses and hemicelluloses in the straw: at the end of 405 days there was left in the case of the corn stalks 3.27–3.83 per cent of the celluloses and 12.35–13.27 per cent of the hemicelluloses; however, in the case of the straw, there was left, at the end of practically the same period of time (386 days) 37.28–48.71 per cent of the celluloses and 47.09 to 53.22 per cent of the hemicelluloses present in the original material.

The lack of available nitrogen was chiefly responsible for the slow decomposition of the rye straw, especially of the celluloses and hemicelluloses. The amount of inorganic nitrogen added to some of the pots was not sufficient to produce conditions most favorable for decomposition of the straw. It resulted only in a somewhat greater reduction of both groups of carbohydrates than in the straw not receiving any additional available nitrogen.

Here also the lignins proved to be more resistant to decomposition than the other constituents of the straw. Likewise, the reduction in total organic matter was less than the decrease of celluloses and hemicelluloses. The total organic matter was reduced by 38.3 per cent in the absence of additional nitrogen and by 44.8 per cent in its presence. However, the celluloses were reduced by 51.3 and 62.7 per cent and the hemicelluloses by 46.8 and 52.9

TABLE 10

Total decomposition of the various organic chemical constituents of rye straw, in the presence of added nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC MATTER LEFT, AFTER DAYS OF DECOMPOSITION | | | | | |
|---------------------------------------|-------------------|--|----------------------|---------------|----------------------|---------------|----------------------|
| | | 66 | | 143 | | 386 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | |
| Total dry material..... | 277.00 | 221.00 | 79.78 | 170.00 | 61.37 | 153.00 | 55.23 |
| Ether-soluble-fraction..... | 5.10 | 2.03 | 39.80 | 1.94 | 38.04 | 0.90 | 17.65 |
| Cold-water-soluble organic matter.... | 12.49 | 4.53 | 36.27 | 3.76 | 30.10 | 2.69 | 21.54 |
| Hot-water-soluble organic matter.... | 4.85 | 4.18 | 86.19 | 3.08 | 63.51 | 2.75 | 56.70 |
| Hemicelluloses..... | 58.42 | 43.34 | 74.19 | 35.87 | 61.40 | 27.51 | 47.09 |
| Celluloses..... | 106.66 | 74.21 | 69.58 | 52.73 | 49.44 | 39.76 | 37.28 |
| Lignins..... | 40.51 | 40.00 | 98.74 | 31.08 | 76.72 | 28.20 | 69.61 |
| Crude protein..... | 2.25 | 4.97 | 220.88 | 4.05 | 180.00 | 5.40 | 240.00 |

per cent respectively. In other words, these two groups of carbohydrates, which make up 60 per cent of the total constituents of the straw, diminished to a considerably greater extent than did the total organic matter. This reduction was balanced by the greater resistance of the lignins to decomposition and by the building up of protein material, a phenomenon already observed as a result of decomposition of the corn stalks.

Because of the comparatively greater abundance of available carbohydrates (celluloses and hemicelluloses), the microorganisms have brought about in the case of the rye straw even a greater proportional increase in the protein content than that which resulted from the decomposition of the corn stalks. This increase was naturally more marked in the presence of additional available nitrogen. The actual increase in the protein content of the residual material was 100 and 140 per cent, without and with the addition of nitrogen, respectively. The increase in the protein content of the decomposing straw

without additional inorganic nitrogen is found to be largely at the expense of the water-soluble nitrogen compounds, which were thereby made water-insoluble. This is brought out in table 11. With the progress of decomposition, there was a gradual decrease of the nitrogen soluble in cold water and a corresponding increase in the nitrogen hydrolyzable by dilute hydrochloric acid and in the more resistant or so-called "humin" nitrogen, pointing to the building up by microorganisms of proteins and other complex organic nitrogenous compounds. Practically all the nitrogen was recovered in the residual

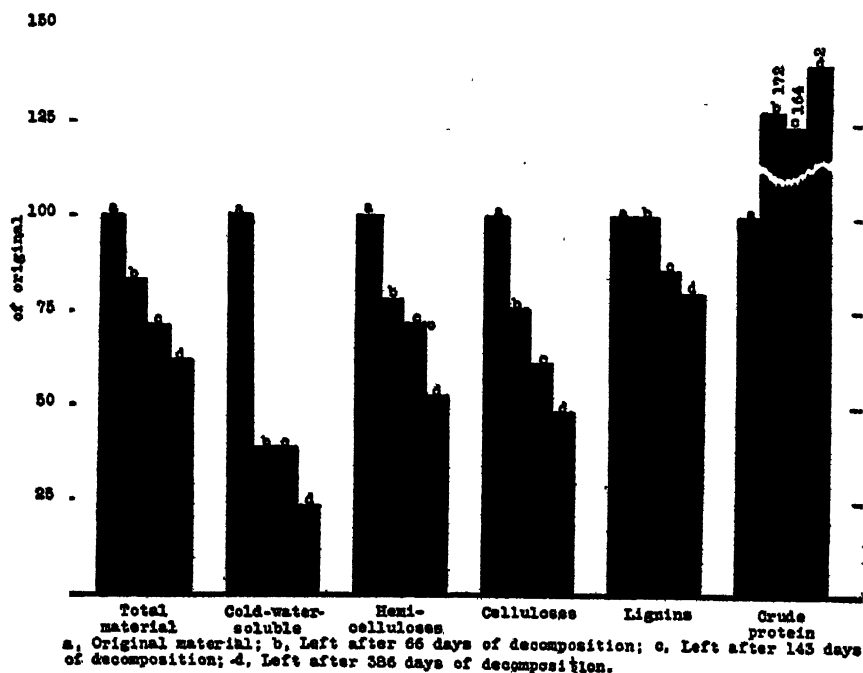


FIG. 3. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF RYE STRAW WITHOUT ADDITIONAL NUTRIENT SALTS

material, pointing to three interesting considerations: (a) there is no loss in nitrogen, either by volatilization or reduction processes, as long as there is sufficient available energy for the activities of microorganisms; (b) there is no gain in nitrogen, through processes of non-symbiotic fixation, when only celluloses and hemicelluloses are available as sources of energy even in the presence of numerous cellulose-decomposing organisms; (c) at least some of the nitrogenous compounds synthesized by the microorganisms are only slowly available sources of nitrogen for soil microorganisms, otherwise the celluloses and hemicelluloses would have undergone a much more rapid decomposition.

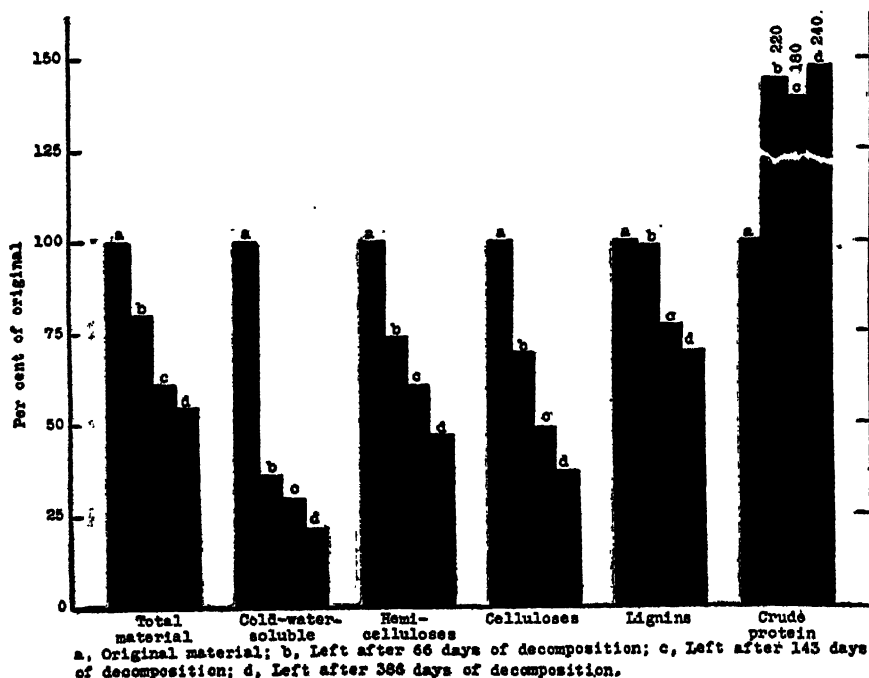


FIG. 4. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF RYE STRAW WITH ADDITIONAL NUTRIENT SALTS

TABLE 11
Nitrogen transformation in the decomposition of rye straw
In per cent of total material

| FORM OF NITROGEN | NO ADDITIONAL NITROGEN | | | | AMMONIUM PHOSPHATE ADDED | | | |
|---|------------------------|---------|----------|----------|--------------------------|---------|----------|----------|
| | Incubation | | | | Incubation | | | |
| | 0 days | 66 days | 143 days | 386 days | 0 days | 66 days | 143 days | 386 days |
| Total nitrogen | 0.24 | 0.33 | 0.39 | 0.50 | 0.31 | 0.42 | 0.47 | 0.64 |
| Soluble in cold water | 0.11 | 0.03 | 0.05 | 0.02 | 0.18 | 0.03 | 0.05 | 0.02 |
| Soluble in hot water | 0 | 0.03 | 0.04 | 0.04 | 0 | 0.03 | 0.05 | 0.05 |
| Hydrolizable by 2 per cent HCl | 0.04 | 0.08 | 0.10 | | 0.04 | 0.12 | 0.14 | |
| "Humin" nitrogen, not acted upon by autoclaving with hot 6 per cent H ₂ SO ₄ .. | 0.06 | 0.10 | 0.13 | | 0.06 | 0.10 | 0.14 | |

DECOMPOSITION OF OAK LEAVES

The mature oak leaves were distinctly different in chemical composition from the corn stalks and the rye straw. The leaves were characterized by a high content of fats and waxes (ether-soluble fraction), a very high lignin (and

cutin) content, and were rich in materials soluble in hot water (tannins). On the other hand, the celluloses and hemicelluloses were not very abundant in the leaves. Their nitrogen content was higher than that of either the corn stalks or the straw. One would expect, therefore, that the leaves should show a decided difference in the nature and rapidity of decomposition from that of the two plant materials previously reported.

TABLE 12

Chemical composition of mature oak leaves at different stages of decomposition, without the addition of nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | |
|-------------------------|-------------------------|-----------------------------|-------|-------|
| | | 66 | 143 | 386 |
| Ether-soluble..... | 3.71 | 3.53 | 2.82 | 1.77 |
| Cold-water-soluble..... | 8.28 | 2.02 | 1.15 | 1.69 |
| Hot-water-soluble..... | 5.65 | 3.91 | 2.15 | 2.16 |
| Hemicelluloses..... | 12.93 | 12.10 | 13.03 | 12.27 |
| Celluloses..... | 13.78 | 11.78 | 10.41 | 8.92 |
| Lignins..... | 30.30 | 40.02 | 42.65 | 44.50 |
| Crude protein..... | 4.25 | 6.00 | 6.13 | 7.44 |
| Ash..... | 5.09 | 6.50 | 6.90 | 8.12 |

TABLE 13

Chemical composition of mature oak leaves at different stages of decomposition, with the addition of nutrient salts

On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | |
|-------------------------|-------------------------|-----------------------------|-------|-------|
| | | 66 | 143 | 386 |
| Ether-soluble..... | 3.71 | 2.65 | 1.74 | 0.67 |
| Cold-water-soluble..... | 8.28 | 1.60 | 1.44 | 2.86 |
| Hot-water-soluble..... | 5.65 | 3.10 | 2.04 | 2.44 |
| Hemicelluloses..... | 12.93 | 12.59 | 13.27 | 13.25 |
| Celluloses..... | 13.78 | 11.90 | 10.94 | 9.23 |
| Lignins..... | 30.30 | 40.40 | 40.55 | 40.35 |
| Crude protein..... | 4.25 | 6.62 | 6.88 | 8.25 |
| Ash..... | 5.09 | 8.00 | 9.00 | 10.33 |

The chemical composition of the oak leaves at different stages of decomposition is given in tables 12 and 13, and the actual concentration of the different organic chemical complexes which have undergone decomposition is recorded in tables 14 and 15 and figures 5 and 6. In the case of this plant material as well, half of the pots received inorganic salts including nitrogen and half did not. The composts of oak leaves were allowed to incubate under the same conditions as the other two plant materials and analyzed after 66, 143, and 386 days.

The results show that although the leaves contained more nitrogen (0.77 per cent) than did the corn stalks, their decomposition was considerably slower. It was similar to that of the rye straw, which contained only about one-third or less of the nitrogen content of the leaves. These differences in the rate

TABLE 14

Total decomposition of the various organic constituents in mature oak leaves, without the addition of nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC SUBSTANCES LEFT, AFTER DAYS OF DECOMPOSITION | | | | | |
|--|-------------------|--|----------------------|---------------|----------------------|---------------|----------------------|
| | | 66 | | 143 | | 386 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | |
| Total dry material | 223.00 | 173.00 | 77.58 | 158.00 | 70.85 | 129.00 | 57.85 |
| Ether-soluble fraction | 8.27 | 6.11 | 73.88 | 4.46 | 53.93 | 2.28 | 27.57 |
| Cold-water-soluble organic matter | 18.46 | 3.49 | 18.91 | 1.82 | 9.86 | 2.18 | 11.81 |
| Hot-water-soluble organic matter | 12.60 | 6.76 | 53.66 | 3.40 | 26.98 | 2.79 | 22.14 |
| Hemicelluloses | 28.83 | 20.93 | 72.54 | 20.59 | 71.39 | 15.83 | 54.91 |
| Celluloses | 30.73 | 20.37 | 66.34 | 16.45 | 53.54 | 11.51 | 37.44 |
| Lignins | 67.56 | 69.23 | 102.47 | 67.39 | 99.75 | 57.41 | 84.97 |
| Crude protein | 4.25 | 10.38 | 244.24 | 9.69 | 228.00 | 9.60 | 225.88 |

TABLE 15

Total decomposition of the various organic constituents in mature oak leaves, in the presence of added nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC SUBSTANCES LEFT, AFTER DAYS OF DECOMPOSITION | | | | | |
|--|-------------------|--|----------------------|---------------|----------------------|---------------|----------------------|
| | | 66 | | 143 | | 386 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | |
| Total dry material | 223.00 | 171.00 | 76.68 | 157.00 | 70.40 | 125.00 | 56.05 |
| Ether-soluble fraction | 8.27 | 4.53 | 54.78 | 2.73 | 33.01 | 0.84 | 10.16 |
| Cold-water-soluble organic matter | 18.46 | 2.74 | 14.84 | 2.26 | 12.24 | 3.58 | 19.39 |
| Hot-water-soluble organic matter | 12.60 | 5.30 | 42.06 | 3.20 | 25.40 | 3.05 | 24.21 |
| Hemicelluloses | 28.83 | 21.53 | 74.63 | 20.83 | 72.20 | 16.56 | 57.44 |
| Celluloses | 30.73 | 20.34 | 66.23 | 17.18 | 55.91 | 11.54 | 37.55 |
| Lignins | 67.56 | 68.48 | 101.36 | 63.66 | 94.23 | 50.44 | 74.66 |
| Crude protein | 4.25 | 11.32 | 266.35 | 10.80 | 254.12 | 10.31 | 242.59 |

and degree of decomposition of the three plant materials lead us to the conclusion that the abundance of nitrogen in the plant residue undergoing decomposition is not the only controlling factor in the rapidity with which microorganisms are capable of attacking it. Actually, the addition of avail-

able nitrogen had no appreciable influence in hastening the decomposition of the leaves, as seen by the reduction in the total organic matter, as well as the celluloses and hemicelluloses.

It has been found, in experiments reported previously (15), that the addition of a small amount of available nitrogen had a decidedly favorable effect upon the decomposition of mature oak leaves, as measured by the evolution of carbon dioxide, the absorption of available nitrogen, and the decomposition of total organic matter, celluloses, and hemicelluloses. The fact, however,

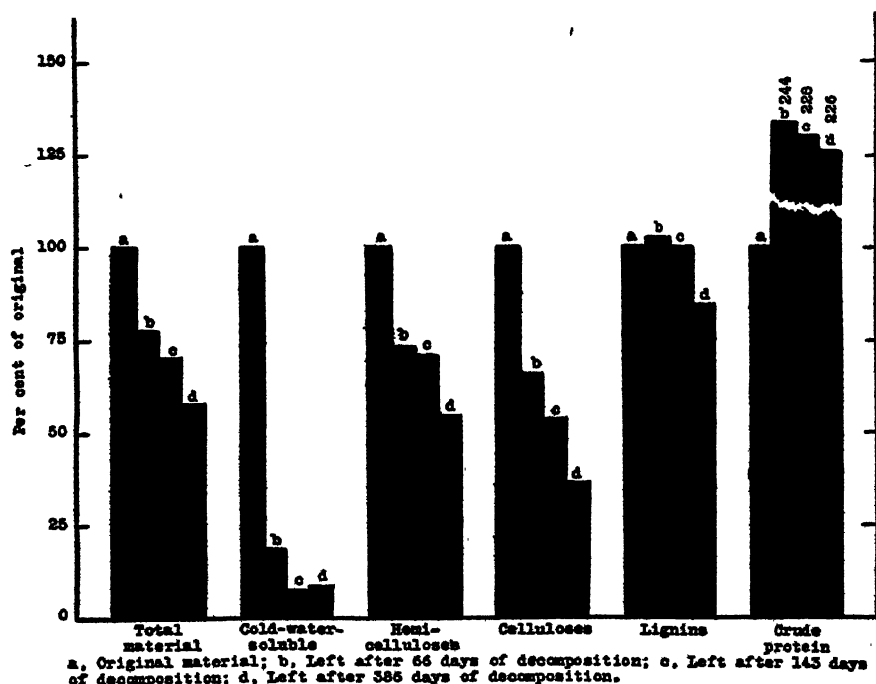


FIG. 5. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF OAK LEAVES WITHOUT ADDITIONAL NUTRIENT SALTS

that in these experiments no such striking differences have been found, do not indicate a possible discrepancy between these two sets of data. The difference is due entirely to the fact that in the previous investigations only a short period of incubation (28 days) was employed; in these studies, however, the first analysis was made only after 68 days of incubation. In other words, even in the case of a plant material with 0.77 per cent nitrogen, the addition of a small amount of available nitrogen will exert a stimulating effect upon its decomposition during the early stages. However, when decomposition has advanced, there will be sufficient nitrogen liberated from the degradation of the plant proteins to take care of the celluloses and hemicelluloses. This

is true especially in the case of a material like oak leaves, the decomposition of which, due to the high lignin, tannin, and wax contents, is considerably delayed, when compared with that of straw and corn stalks. Even in the early periods of incubation, the effect of additional nitrogen upon the decomposition of the oak leaves was considerably less than upon the decomposition of rye straw and corn stalks, as shown in the earlier contribution (15).

Although the water-soluble substances of the oak leaves decomposed very rapidly, the celluloses and hemicelluloses resisted decomposition more than

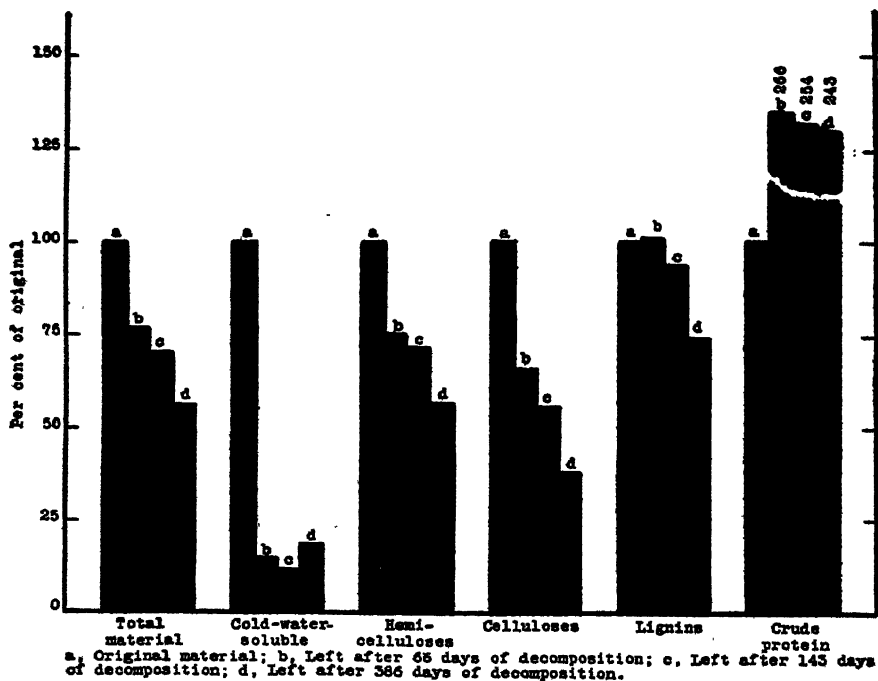


FIG. 6. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF OAK LEAVES WITH ADDITIONAL NUTRIENT SALTS

in the case of the two other plant materials. This is due, not to the lack of the available nitrogen, as found in the case of the rye straw, but to the abundance of the lignins. It has been shown elsewhere (13) that the lignins are not only resistant to decomposition, but they even delay the disintegration of the celluloses. The removal of the lignins from a plant residue hastens the decomposition of the celluloses. In the case of the oak leaves, the lignins remained practically undecomposed, until a year had passed, and only then a small fraction had disappeared, the nature of the change still remaining undetermined.

The crude or water-insoluble proteins have also increased in the case of the

oak leaves. Where nitrogen was added in the form of ammonia, it was changed rapidly into organic nitrogen by the synthetic action of the microorganisms. When no inorganic nitrogen was added, the water-soluble nitrogen compounds disappeared, giving rise to water-insoluble nitrogenous complexes. This is brought out in table 16.

When the results of the decomposition of mature oak leaves are compared with that of green oak leaves, as reported elsewhere (16), marked differences are observed, due entirely to differences in the chemical composition of the two kinds of leaves used in both experiments. The younger leaves were richer in water-soluble constituents and in nitrogen, and poorer in lignins. As a result of these differences, the younger leaves decomposed much more rapidly, large amounts of nitrogen actually becoming liberated in the form

TABLE 16
Nitrogen transformation in the decomposition of oak leaves
In per cent of total material

| FORM OF NITROGEN | NO ADDITIONAL NITROGEN | | | | AMMONIUM PHOSPHATE ADDED | | | |
|--|------------------------|---------|----------|----------|--------------------------|---------|----------|----------|
| | Incubation | | | | Incubation | | | |
| | 0 days | 66 days | 143 days | 386 days | 0 days | 66 days | 143 days | 386 days |
| Total nitrogen..... | 0.77 | 1.03 | 1.06 | 1.26 | 0.87 | 1.15 | 1.20 | 1.43 |
| Soluble in cold water..... | 0.09 | 0.02 | 0.04 | 0.03 | 0.19 | 0.04 | 0.05 | 0.04 |
| Soluble in hot water..... | Trace | 0.05 | 0.04 | 0.05 | Trace | 0.05 | 0.05 | 0.07 |
| Hydrolizable by 2 per cent HCl..... | 0.18 | 0.17 | 0.22 | | 0.18 | 0.21 | 0.27 | |
| "Humin" nitrogen, not acted upon by autoclaving with 6 per cent H ₂ SO ₄ | 0.30 | 0.47 | 0.53 | 0.49 | 0.30 | 0.49 | 0.53 | 0.53 |

of ammonia even within a period of six months of decomposition under aerobic conditions.

DECOMPOSITION OF ALFALFA

The chemical composition of the alfalfa plant used in these experiments differed considerably from that of the other three plant materials. As a representative of the group of leguminous plants, it was characterized by a high nitrogen content, a large part of which was water-soluble. It contained also the largest amount of cold-water-soluble substances, which is the fraction previously found to disappear most rapidly when a plant is undergoing decomposition. The alfalfa also contained the lowest lignin content; this would tend to favor further the ease of its decomposition. One would expect from an analysis of the chemical composition of this material that it should undergo rapid disintegration, especially during the early stages.

It has been shown previously (15), that when a plant material contains about 1.7 per cent nitrogen it undergoes rapid decomposition even when no

additional nitrogen is available. In view of the fact that the alfalfa contained more than this amount of nitrogen, the addition of inorganic nitrogen should not exert any favorable effect upon the rapidity of its decomposition. This was actually found to be the case. The decomposing alfalfa receiving ammonium phosphate and calcium carbonate actually decomposed more

TABLE 17
Chemical composition of alfalfa at different stages of decomposition, without the addition of nutrient salts
On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | | |
|-------------------------|-------------------------|-----------------------------|-------|-------|-------|
| | | 27 | 68 | 205 | 405 |
| Ether-soluble..... | 2.75 | | 1.90 | 0.71 | 0.90 |
| Cold-water-soluble..... | 12.44 | 12.55 | 11.08 | 9.16 | 11.15 |
| Hot-water-soluble..... | 4.80 | 5.13 | 7.13 | 6.50 | 6.34 |
| Hemicelluloses..... | 8.52 | 6.24 | 6.39 | 7.29 | 8.22 |
| Celluloses..... | 26.71 | 22.63 | 16.48 | 15.15 | 15.90 |
| Lignins..... | 10.78 | 15.12 | 14.85 | 18.37 | 16.08 |
| Crude protein..... | 8.13 | 8.43 | 7.31 | 10.31 | 9.18 |
| Ash..... | 10.30 | | | 23.43 | 24.88 |

TABLE 18
Chemical composition of alfalfa at different stages of decomposition, with the addition of nutrient salts
On per cent basis of dry material

| CHEMICAL CONSTITUENTS | ORIGINAL PLANT MATERIAL | AFTER DAYS OF DECOMPOSITION | | | |
|-------------------------|-------------------------|-----------------------------|-------|-------|-------|
| | | 27 | 68 | 205 | 405 |
| Ether-soluble..... | 2.75 | | 2.02 | 1.08 | 0.60 |
| Cold-water-soluble..... | 12.44 | 10.10 | 10.87 | 8.23 | 9.81 |
| Hot-water-soluble..... | 4.80 | 4.34 | 5.56 | 5.94 | 6.69 |
| Hemicelluloses..... | 8.52 | 6.15 | 6.19 | 8.07 | 7.16 |
| Celluloses..... | 26.71 | 26.00 | 19.31 | 17.22 | 17.55 |
| Lignins..... | 10.78 | 14.91 | 16.51 | 16.98 | 15.62 |
| Crude protein..... | 8.13 | 7.94 | 8.50 | 12.25 | 8.56 |
| Ash..... | 10.30 | | | 25.54 | 27.88 |

slowly than the material not receiving any additional salts. This can be explained as follows: the rapid decomposition of the alfalfa, rich in soluble nitrogen, led to an abundant liberation of ammonia. Because the compost was enclosed in a covered pot, the reaction became alkaline. The addition of ammonium phosphate and calcium carbonate tended to make the reaction even more alkaline, so that the ammonia actually saturated the atmosphere of the compost; this resulted in a considerable depression of many groups of organisms, notably of the fungi and various bacteria. This depression had a

TABLE 19

Total decomposition of the various organic constituents of alfalfa, without the addition of nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC SUBSTANCES LEFT, AFTER DAYS OF DECOMPOSITION | | | | | | | |
|--|-------------------|--|----------------------|---------------|----------------------|---------------|----------------------|---------------|----------------------|
| | | 27 | | 68 | | 205 | | 405 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | | gm. | |
| Total dry material..... | 260.00 | 181.00 | 70.00 | 151.80 | 58.40 | 119.10 | 45.80 | 100.00 | 38.50 |
| Ether-soluble fraction.... | 7.15 | | | 2.88 | 40.28 | 0.85 | 11.89 | 0.90 | 12.59 |
| Cold-water-soluble organic matter..... | 32.34 | 22.72 | 70.25 | 16.82 | 52.01 | 10.91 | 33.74 | 11.15 | 34.48 |
| Hot-water-soluble organic matter..... | 12.48 | 9.29 | 74.44 | 10.82 | 86.70 | 7.74 | 62.02 | 6.34 | 50.80 |
| Hemicelluloses..... | 22.16 | 11.29 | 50.94 | 9.70 | 43.77 | 8.68 | 39.17 | 8.22 | 37.09 |
| Celluloses..... | 69.45 | 40.95 | 58.96 | 25.02 | 36.03 | 18.04 | 25.98 | 15.90 | 22.89 |
| Lignins..... | 28.02 | 27.37 | 97.68 | 22.54 | 80.44 | 21.88 | 78.09 | 16.08 | 57.39 |
| Crude protein..... | 21.14 | 15.27 | 72.23 | 11.10 | 52.51 | 12.28 | 58.09 | 9.18 | 43.42 |

TABLE 20

Total decomposition of the various organic constituents of alfalfa, with the addition of nutrient salts

| ORGANIC CONSTITUENTS | ORIGINAL MATERIAL | ORGANIC SUBSTANCES LEFT, AFTER DAYS OF INCUBATION | | | | | | | |
|--|-------------------|---|----------------------|---------------|----------------------|---------------|----------------------|---------------|----------------------|
| | | 27 | | 68 | | 205 | | 405 | |
| | | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original | Total residue | Per cent of original |
| | gm. | gm. | | gm. | | gm. | | gm. | |
| Total dry material..... | 260.00 | 207.00 | 79.62 | 151.89 | 58.40 | 116.00 | 44.50 | 109.80 | 42.20 |
| Ether-soluble fraction.... | 7.15 | | | 3.07 | 42.94 | 1.25 | 17.48 | 0.66 | 9.23 |
| Cold-water-soluble organic matter..... | 32.34 | 20.71 | 64.04 | 16.51 | 51.05 | 9.55 | 29.53 | 10.77 | 33.30 |
| Hot-water-soluble organic matter..... | 12.48 | 8.90 | 71.31 | 8.44 | 67.63 | 6.89 | 55.21 | 7.35 | 58.89 |
| Hemicelluloses..... | 22.16 | 12.60 | 56.86 | 9.40 | 42.42 | 9.36 | 42.24 | 7.86 | 35.47 |
| Celluloses..... | 69.45 | 53.30 | 76.75 | 29.32 | 42.22 | 19.98 | 28.77 | 19.27 | 27.75 |
| Lignins..... | 28.02 | | | 25.07 | 89.47 | 19.70 | 70.31 | 17.15 | 61.21 |
| Crude protein..... | 21.14 | 16.28 | 77.01 | 12.91 | 61.07 | 14.21 | 67.22 | 9.40 | 44.47 |

marked effect upon the rapidity of decomposition of some of the organic complexes in the alfalfa, especially of the celluloses.

Tables 17 and 18 give the chemical composition of the alfalfa at the different stages of decomposition. Analyses were made after 27, 68, 205, and 405 days of incubation at 25–27°C. Tables 19 and 20 and figures 7 and 8 give the total amounts of the various chemical constituents in the residual matter, at the different periods of incubation.

A comparison of the rapidity of decomposition of corn stalks, rye straw, and alfalfa, as measured by the evolution of carbon dioxide, has shown (15)

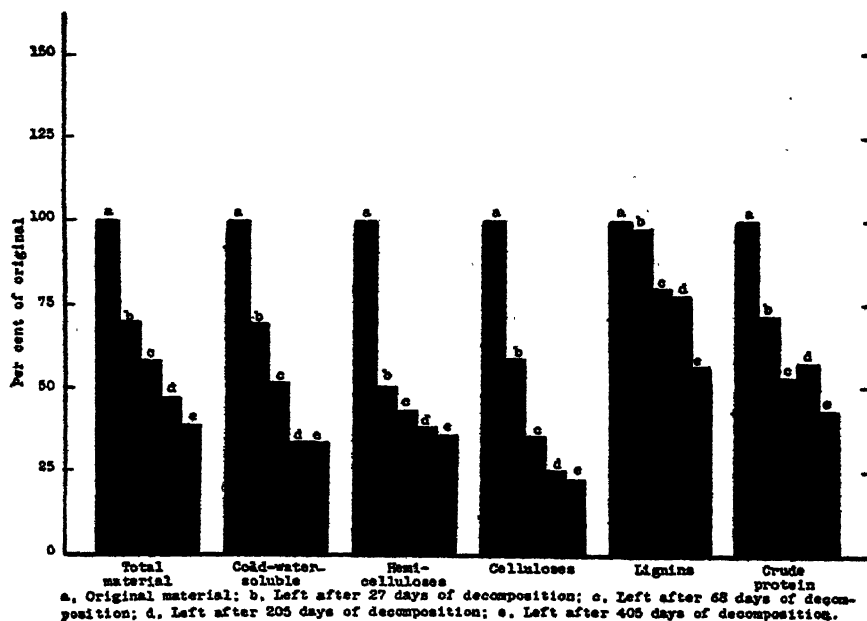


FIG. 7. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF ALFALFA WITHOUT ADDITIONAL NUTRIENT SALTS

that the alfalfa decomposed far more rapidly than did the other two materials. However, when a long period of incubation is used, the alfalfa is found to leave a considerably larger amount of residual material than do the corn stalks. This is due primarily to the artificial conditions under which these studies were carried out. The rapid accumulation of ammonia as a result of the protein decomposition in the nitrogen-rich alfalfa prevented a rapid development of the fungi and various bacteria, which accounts for the larger amount of celluloses and hemicelluloses that remained undecomposed than in the case of the corn stalks.

The lignins of the alfalfa were also more resistant to decomposition than the other organic complexes. Although the relative protein content of the

compost tended to increase with the advance of decomposition, especially within the first 205 days, the total protein content diminished rapidly. This

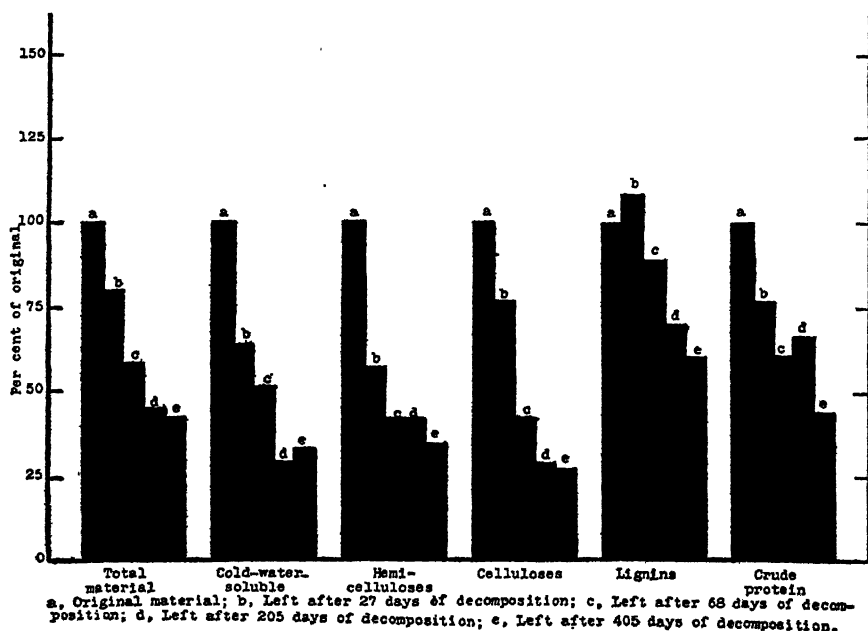


FIG. 8. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF ALFALFA WITH ADDITIONAL NUTRIENT SALTS

TABLE 21

Nitrogen transformation in the aerobic decomposition of alfalfa
In per cent of total material

| FORM OF NITROGEN | NO ADDITIONAL NITROGEN | | | | | AMMONIUM PHOSPHATE ADDED | | | | |
|---|------------------------|---------|---------|----------|----------|--------------------------|---------|---------|----------|----------|
| | Incubation | | | | | Incubation | | | | |
| | 0 days | 27 days | 68 days | 205 days | 405 days | 0 days | 27 days | 68 days | 205 days | 405 days |
| Total nitrogen..... | 2.58 | 2.63 | 2.70 | 2.60 | 2.40 | 2.66 | 2.32 | 2.47 | 2.80 | 2.32 |
| Ammonia nitrogen..... | 0 | 0.39 | 1.66 | | | 0.08 | 0.51 | 1.68 | | |
| Soluble in cold water..... | 0.93 | 0.98 | 0.78 | 0.57 | 0.61 | 1.01 | 0.79 | 0.75 | 0.59 | 0.65 |
| Soluble in hot water..... | 0.35 | 0.30 | 0.39 | 0.38 | 0.32 | 0.35 | 0.26 | 0.36 | 0.35 | 0.30 |
| Hydrolyzable in 2 per cent HCl..... | 0.59 | 0.38 | 0.47 | | 0.62 | 0.59 | 0.37 | 0.51 | | 0.57 |
| "Humin" nitrogen, not acted upon by autoclaving with 6 per cent H ₂ SO ₄ | 0.37 | 0.45 | 0.41 | 0.39 | 0.34 | 0.37 | 0.42 | 0.49 | 0.47 | 0.38 |

is the opposite of what has taken place in the case of the other three plant materials and is due to the initial high protein content of the alfalfa. This is brought out in table 21. The total nitrogen remained at about the same

level, whereas the water-soluble nitrogen disappeared only partly. There was also no marked accumulation of proteins readily hydrolyzable by dilute (2 per cent) hydrochloric acid and of the non-hydrolyzable, so-called "humin" nitrogen. These facts are again distinctly different from those which have been found in the case of the nitrogen-poor plant materials.

DISCUSSION

An analysis of the data dealing with the decomposition of four different plant materials, varying markedly in origin and in chemical composition, brings out some illuminating facts. These can be summarized as follows: *the rapidity and nature of decomposition of plant residues under aerobic conditions depend primarily upon the chemical composition of the particular plant materials.*

Among the various chemical constituents of the plant materials which may influence their decomposition, the following deserve careful consideration:

1. The amount and nature of the constituents which are soluble in cold water.
2. The abundance of the celluloses and hemicelluloses.
3. The amount and nature of the nitrogenous complexes.
4. The abundance of the lignins.

The greater the amount of water-soluble material in a plant residue the more rapid will be its decomposition, especially during the early stages. This is easy to understand, since the water-soluble fraction comprises the simple sugars, amino acids, soluble salts,—substances all readily used by a great number of microorganisms, both fungi (especially the Phycomycetes) and bacteria (especially the spore-forming organisms). The younger a plant is, the higher is its content of water-soluble substances. The decrease in the rapidity of decomposition with the increase in age of plant is due, among other factors, to a decrease in this group of plant constituents. Further, the decomposition of sugars, organic acids, and other water-soluble organic complexes can be carried on in the soil by both aerobic and anaerobic bacteria, which are capable of using gaseous atmospheric nitrogen; hence, the amount and availability of the nitrogen are not controlling factors in the decomposition of these organic materials.

The most abundant groups of constituents in the various plant residues which undergo decomposition in the soil or in manure are the celluloses and hemicelluloses. Usually they make up about 50 to 65 per cent of the sum total of the dry plant material. Their decomposition is carried out by certain groups of fungi and specific bacteria. These organisms synthesize considerable cell substance in the process of decomposition of the celluloses. For this purpose a certain amount of available nitrogen is required. Hence the decomposition of the celluloses and hemicelluloses depends upon the presence of organisms capable of decomposing them and upon the amount of available nitrogen.

The celluloses are usually not present in the plant residues in a free condition but are combined largely with lignins, in the form of ligno-celluloses. Whether the combination is chemical or physical in nature, the presence of lignin markedly influences the ability of the microorganisms to decompose the celluloses. The various chemical treatments which have in view an increase of digestibility by animals of plant residues high in lignins consist in the partial dissolution of the lignins. The lignins themselves are decomposed by microorganisms (with certain few exceptions, as in the case of some wood-destroying Hymenomycetes) only to a very limited extent, even under aerobic conditions. A long time may elapse before any measurable reduction in the lignins will be observed.

It must be kept in mind that the decomposition processes taking place in these studies were carried out at an optimum temperature and moisture; the conditions were also favorable for the activities of aerobic organisms. But even under these conditions, the decomposition of the lignins has been very limited in comparison with that of the celluloses and other organic complexes. In the case of the corn stalks with nutrients, there was left, after 405 days of decomposition, 20.30 per cent of the total organic matter, 3.83 per cent of the cellulose, and 42.49 per cent of the lignin. In the case of the rye straw, there was left, after 386 days incubation, 55.23 per cent of the total organic matter, 37.28 per cent of the cellulose, and 69.61 per cent of the lignin. In the case of the oak leaves, there was left, after 386 days decomposition, 56.05 per cent of the total organic matter, 37.55 per cent of the cellulose, and 74.66 per cent of the lignin. In the case of the alfalfa, there was left, after 405 days of decomposition, 42.20 per cent of the total organic matter, 27.75 per cent of the cellulose, and 61.21 per cent of the lignin. In other words, all the four plant materials, obtained from different sources and varying markedly in chemical composition, behaved alike as far as the lignins were concerned. The reduction of the lignins was considerably less than that of the total plant residues and much less than any of the other organic plant constituents.

The behavior of the nitrogen complexes deserves also special consideration. The rapid increase in the protein content as a result of the decomposition of plant residues with a low nitrogen content has been definitely established. That this protein is a result of the synthesizing activities of the microorganisms and that a definite ratio exists between the amount of cellulose decomposed and the nitrogen transformed from an inorganic into an organic form has been amply demonstrated previously. On the other hand, the results on the decomposition of alfalfa, a plant residue with a nitrogen content greater than 1.7 per cent, showed a decrease of the total protein, although accompanied at the same time by an increase in the relative nitrogen content of the decomposing material. In other words, even in the case of a protein-rich plant material, the processes of decomposition were accompanied by synthesis of new proteins, so that the nitrogenous complexes which resulted

from the decomposition of the alfalfa are of an entirely different nature from the original plant proteins.

Among the other organic constituents of the plant residues undergoing decomposition, the hot-water-soluble fraction deserves some consideration. This fraction has decreased to a less extent than many of the other groups of plant complexes. It is quite distinct from the cold-water-soluble fraction in that it is reduced much less rapidly than the latter. This is due to two factors: (a) Hot water extracts certain complexes, like the tannins, which are quite resistant to decomposition; (b) the microorganisms synthesize various substances soluble in hot water but not in cold water.

The ether-soluble fraction comprises also a heterogeneous group of complexes, including various oils, fats, waxes, and sterols. Some of these decompose quite rapidly, whereas others are very resistant to decomposition. This is largely the reason why the ether-soluble fraction of alfalfa or corn stalks decomposed readily whereas that of the straw proved to be much more resistant to decomposition.

SUMMARY

1. The decomposition of four plant materials; namely, corn stalks, rye straw, oak leaves, and alfalfa plants, has been studied under aerobic conditions, in the presence of an optimum amount of moisture and at 25–27°C.

2. The processes of decomposition have been followed by determining the disappearance of the total organic matter as well as of the more important groups of organic complexes.

3. The nature and rapidity of decomposition of different plant materials are markedly influenced by their chemical composition, provided the conditions for decomposition are the same.

4. The decomposition of the sugars, celluloses, hemicelluloses, fats, and proteins account for most of the decomposed plant materials.

5. The lignins are more resistant to decomposition and tend, therefore, to accumulate. Under aerobic conditions, however, there is a decided reduction in the total lignin content, indicating that, although it is attacked less readily than the celluloses and proteins, its resistance to decomposition is only relative.

6. In the case of plant materials with a low nitrogen content (0.2 to 1.7 per cent), the decomposition of the organic matter is accompanied by a relative and absolute increase in the crude protein content. This is due to the synthesizing activities of the microorganisms, which obtain their energy from the decomposition of the celluloses and hemicelluloses.

7. The celluloses and hemicelluloses, including the pentosans, disappear more rapidly than can be accounted for by the reduction in total organic matter. This is balanced by the accumulation of the lignins and proteins.

8. Among the other synthesized products, the hemicelluloses occupy a prominent place. This is partly accounted for by the fact that, although the pentosans comprising most of the hemicelluloses in the plant material begin

to decompose more rapidly than the celluloses, toward the end of decomposition there is left more hemicellulose than cellulose.

9. The addition of available inorganic nitrogen salt hastens the decomposition of celluloses and hemicelluloses, hence of those plant materials which are rich in celluloses and hemicelluloses and poor in proteins.

10. The residues left, after the plant materials have decomposed for a period of 12 to 14 months at an optimum temperature and moisture and under aerobic conditions, possess all the properties of soil organic matter or soil "humus." This is true especially of the corn stalks which have undergone most rapid decomposition.

11. This residual organic matter or "humus" is made up chiefly of lignins or modified lignin complexes largely of plant origin, of proteins and other complex organic nitrogenous compounds largely of microbial origin, of hemicelluloses partly of plant and partly of microbial origin, of a small amount of celluloses undergoing decomposition, and of varying concentrations of a number of different other organic complexes either in the process of decomposition, resistant to decomposition, or products of decomposition.

12. This residual material or "humus" is not in a state of equilibrium but undergoes continuous change, the rate of change becoming constantly slower, approaching that of the soil "humus."

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THE AVAILABILITY OF NITROGENOUS FERTILIZERS TO RICE¹

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The problem of maintaining an adequate supply of available nitrogen in soils for the nutrition of rice offers many difficulties because of the anaerobic condition caused by the irrigation of the crop. According to the generally accepted conception of changes taking place in the available nitrogen, excluding the assimilation by crops under anaerobic conditions, the nitrate nitrogen may be reduced to ammonia or free nitrogen. Depending upon the organisms and environment, the available nitrogen may be used directly as food by bacteria, and the ammonia from ammonium compounds may be adsorbed by the base exchange complexes in the soil. A material decrease in the available nitrogen by any one or more of the above processes might cause a decrease in the yield of rice.

With the present trend of agriculture being to increase yields per acre, the proper fertilization of crops is becoming a leading problem. Unfortunately the fertilization of rice has not proved profitable in many instances. Particularly is this true in Arkansas where the increased yield, if any, has often failed to pay for the cost of the fertilizer. The abnormal condition produced when the rice fields are irrigated suggests that the problem of maintaining available nitrogen may be one of the first problems affecting crop yields.

Some studies have already been made concerning the availability of some forms of nitrogen for rice but the results are contradictory and do not include some of the new nitrogenous compounds produced during the past few years for commercial fertilizers. Further study under controlled conditions was started to determine the reasons for the discrepancies in the results reported by other workers and to secure additional information on the question of nitrogen fertilization of rice.

The objects of the experiment were: first, to determine the availability of various nitrogenous compounds to rice; second, to study the nitrogen changes taking place under irrigated conditions.

The experiments made by a number of investigators on the availability of nitrogen to rice might be grouped, according to the conclusions drawn, into the three following groups; those showing nitrates and ammonium sulfate

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equally good for the production of rice, those concluding that ammonium compounds gave the best results, and those showing that organic compounds gave the best results.

Among the first experiments having much significance from the standpoint of rice fertilization were those of Kellner (16) in 1882. The conclusion drawn from his experiments was that nitrates alone gave higher yields than ammonium sulfate, although he goes on to state that a mixture of the two compounds gave the best results. Van Rossem (21) from field experiments reports that sodium nitrate plus superphosphate gave larger yields than did ammonium sulfate and superphosphate. Hartenbower (12) at the Guam experiment station found that nitrate of soda gave the largest yield of any single fertilizer treatment and the yield was only exceeded by the complete fertilizer treatment. Similar results have been reported more recently by Briggs (3) working at the same station. De Jong and Van Rossem (7) from a comparison of different sources of nitrogen concluded that calcium nitrate was nearly equal to ammonium sulfate for rice nutrition and that late applications of sodium nitrate gave good results. Willis and Carrero (24) state that if other factors are controlled, rice will give as good results with sodium nitrate as with ammonium sulfate. They do claim, however, that ammonium sulfate is the safest to use because there are not so many factors involved that may affect its availability as there are with sodium nitrate. Rice grown in culture solutions under controlled conditions gave good growth when nitrogen was supplied as sodium nitrate in experiments conducted by Villegas (22). In further studies using sterile cultures he states that rice flourished when potassium nitrate, ammonium sulfate, asparagin, glycolcol, and foramide were used as sources of nitrogen.

At the time the aforementioned experiments were being published, other reports claiming that ammonium sulfate was the best fertilizer for rice, were appearing in the literature. Nagaoka (18) and Daikuhara and Imaseki (6) reported from pot experiments that ammonium sulfate was the more effective fertilizer. Daikuhara and Imaseki state, however, that when rice was grown as a dry land crop, sodium nitrate and ammonium sulfate were about of equal value. Kelley (14) from the results of several years pot and field experiments concluded that ammonium sulfate was far superior to sodium nitrate.

Harrison (10) from field experiments states "Paddy soils need bulky organic manures or ones yielding ammonia under anaerobic conditions." The laterite soils in the rice district of India give good results only with cow manure according to Clouston (5). In southwestern Louisiana (4) the best results were obtained when soybeans were used in a rotation which did not include the use of fertilizers. More recently Janssen and Metzger (13) have shown that green manures may be valuable sources of nitrogen for the fertilization of rice.

With such a mass of conflicting data it seems reasonable to believe that there are certain factors other than the form of nitrogen, due to the nitrogenous compounds used, which control the growth of the plants. A recent report (2)

indicates that pH changes due to the form of nitrogen used may affect the availability of certain forms of nitrogen more than others.

PLAN OF EXPERIMENT

Experiments on the availability to rice of various nitrogenous compounds, were made in sand cultures, fertilized, with insufficient nitrogen for normal growth. Estimations made from results by Kelley (14) indicated that 0.3 gm. would be insufficient for complete normal growth and therefore the plant would utilize all of the available nitrogen. Equivalent amounts of nitrogen, 0.3 gm., were mixed in jars containing 12 kgm. of sand. The nitrogen was added as ammonium sulfate, commercial ammonium phosphate, Leunasal-peter, calcium cyanamid, sodium nitrate, calcium nitrate, urea, cotton seed meal, blood meal, and mixtures having their nitrogen one-half as cotton seed meal and one-half as sodium nitrate; and one-half as cotton seed meal and one-half as ammonium sulfate. Samples of the compounds were saved for analysis in order to calculate the exact amount of nitrogen added to each jar. Unfortunately the ammonium phosphate did not contain the amount thought to be present and the amount added did not carry as much nitrogen as the other treatments. The same amounts of ammonium phosphate were added in all experiments to keep the results comparable. All treatments were made in duplicate. After the rice was planted, a nitrogen-free nutrient solution was added. When the plants were one inch high they were thinned to 12 plants per jar and when they were six inches high they were irrigated with distilled water so that water stood two inches deep over the soil. The reaction was maintained as near pH 6.5 as possible by semi-weekly additions of dilute solutions of hydrochloric acid or sodium hydroxide, as necessary, until during a period of two weeks, constancy of the pH made further additions unnecessary.

During the whole time in which the rice was growing the surface of the sand was kept under water by additions of distilled water as often as was necessary. The rice was harvested shortly after blossoming to prevent any possible loss of nitrogen. Kelley and Thompson (15) and Sen (20) have shown that the intake of nitrogen by rice after the flowering stage is very small. The roots from each jar were washed free from sand. All samples, tops, and roots were first air-dried and then oven-dried and weighed separately. The tops and roots were ground together and thoroughly mixed for determination of total nitrogen.

GROWTH STUDIES

Chemical studies on availability of nitrogenous compounds may give some information in regard to their value as a fertilizer, but growth of plants followed by chemical analyses should give a better estimation of the availability. Two consecutive experiments were made according to the method described. In both experiments, the plants became chlorotic shortly after being flooded and remained so until almost the time of flowering, when they became green again and remained that color until changing due to maturation of the plants. That

this chlorotic condition may have been caused by a deficiency of available nitrogen and not by a lack of soluble iron as suggested by Gile and Carrero (6) will be shown later. In fact, several applications of soluble ferric phosphate and ferric citrate failed to overcome the chlorotic condition.

The average yields of oven-dried material from two jars with the different treatments are given in table 1 for both experiments.

The results on the whole agree very well when it is considered that the weight of dry matter produced is not a good criterion of availability when considered alone. The dry matter produced when urea and ammonium phosphate were used was the same in both experiments and agrees very well with that previously reported (2) in another experiment. Similarly the dry weights from the

TABLE 1
Yields of rice grown in quartz sand with the nitrogen fertilizer indicated

| SOURCE OF NITROGEN | FIRST EXPERIMENT | | | SECOND EXPERIMENT | | |
|--|------------------|-------|---------|-------------------|-------|---------|
| | Tops | Roots | Average | Tops | Roots | Average |
| | gm. | gm. | gm. | gm. | gm. | gm. |
| None..... | 1.8 | 1.5 | 3.3 | 1.2 | 0.7 | 1.9 |
| NaNO ₃ | 20.1 | 15.9 | 36.0 | 13.3 | 12.3 | 25.6 |
| Ca(NO ₃) ₂ | | |* | 10.8 | 10.6 | 21.4 |
| (NH ₄) ₂ SO ₄ | 15.6 | 9.7 | 25.3 | 16.7 | 16.9 | 33.6 |
| (NH ₄) ₂ PO ₄ | 8.2 | 6.5 | 14.7 | 7.8 | 5.2 | 13.0 |
| Urea..... | 17.0 | 14.7 | 31.7 | 12.6 | 12.3 | 24.9 |
| Calcium cyanamid..... | 12.5 | 8.9 | 21.4 | 8.8 | 7.9 | 16.7 |
| Cotton seed meal..... | 9.2 | 9.9 | 19.1 | 12.8 | 11.3 | 24.1 |
| Blood meal..... | 9.9 | 9.7 | 19.6 | 13.3 | 12.4 | 25.7 |
| Leunasalpeter..... | 16.5 | 13.6 | 30.1 | 16.6 | 14.2 | 30.8 |
| Cotton seed meal and NaNO ₃ | | | | 11.5 | 11.8 | 23.3 |
| Cotton seed meal and (NH ₄) ₂ SO ₄ | | | | 18.9 | 15.9 | 34.8 |

* Plants died.

organic treatments agree very well in the two experiments and are very close to the amounts previously reported under reaction studies. The other results vary somewhat, showing sodium nitrate to be superior to ammonium sulfate in the first experiment and inferior in the second experiment. However, the differences in neither case are large enough to indicate that one is greatly superior to the other. The probable explanation is that the two are almost equally efficient and physiological disturbances caused the differences in growth. It is of interest to note that the average yields of the two experiments from Leunasalpeter where the nitrogen is supplied as a mixture of nitrate and ammoniacal nitrogen are as good as those from any other form.

From the standpoint of dry matter produced, calcium nitrate, calcium cyanamid, blood meal, cotton seed meal, and the mixture of cotton seed meal and sodium nitrate gave yields low enough to indicate that the nitrogen contained

was less available than the other forms. In saying this it must be remembered that only half as much nitrogen was added in the ammonium phosphate treatment, and the yields would have to be doubled to be considered, or else left entirely out of consideration. When the amount of dry matter produced with

TABLE 2
Assimilation of nitrogen by rice

| SOURCE OF NITROGEN | N ADDED | FIRST EXPERIMENT | | | SECOND EXPERIMENT | | |
|--|---------|------------------|-------------------------|--------------|-------------------|-------------------------|--------------|
| | | N in plant | N assimilated by plants | Availability | N in plant | N assimilated by plants | Availability |
| | mgm. | per cent | mgm. | per cent | per cent | mgm. | per cent |
| NaNO ₃ | 295.0 | 0.605 | 187.7 | 63.6 | 0.635 | 162.3 | 55.0 |
| CaNO ₃ | 300.0 | | | | 0.510 | 109.0 | 36.4 |
| (NH ₄) ₂ SO ₄ | 302.8 | 0.880 | 218.0 | 71.7 | 0.550 | 185.0 | 61.2 |
| (NH ₄) ₂ PO ₄ | 152.6 | 0.660 | 97.0 | 63.8 | 0.575 | 74.6 | 48.8 |
| Urea..... | 264.0 | 0.525 | 166.5 | 63.1 | 0.605 | 151.5 | 57.2 |
| Calcium cyanamid..... | 300.0 | 0.600 | 128.4 | 42.8 | 0.800 | 143.8 | 48.0 |
| Cotton seed meal..... | 305.5 | 0.615 | 119.7 | 39.1 | 0.515 | 123.9 | 40.6 |
| Blood meal..... | 246.8 | 0.750 | 146.6 | 59.0 | 0.540 | 138.5 | 56.2 |
| Leunasalpeter..... | 296.5 | 0.660 | 198.0 | 67.0 | 0.585 | 180.3 | 60.8 |
| Cotton seed meal plus NaNO ₃ | 300.0 | | | | 0.515 | 121.4 | 40.5 |
| Cotton seed meal plus (NH ₄) ₂ SO ₄ | 304.2 | | | | 0.510 | 177.5 | 58.4 |

TABLE 3
Relative availability of nitrogenous fertilizers for rice, ammonium sulfate considered as 100

| SOURCE OF NITROGEN | RELATIVE AVAILABILITY | | |
|---|-----------------------|-------------------|---------|
| | First experiment | Second experiment | Average |
| NaNO ₃ | 89 | 90 | 89.5 |
| CaNO ₃ | ... | 59 | 59.0 |
| (NH ₄) ₂ SO ₄ | 100 | 100 | 100.0 |
| (NH ₄) ₂ PO ₄ | 89 | 80 | 84.5 |
| Urea..... | 88 | 94 | 92.0 |
| Calcium cyanamid..... | 60 | 79 | 69.5 |
| Cotton seed meal..... | 55 | 66 | 61.5 |
| Blood meal..... | 82 | 92 | 87.0 |
| Leunasalpeter..... | 93 | 99 | 96.0 |
| Cotton seed meal plus NaNO ₃ | ... | 66 | 66.0 |
| Cotton seed meal plus (NH ₄) ₂ SO ₄ | ... | 96 | 96.0 |

half the amount of nitrogen is compared with the other yields, ammonium phosphate appears to be a suitable source of nitrogen for the fertilization of rice. Sodium nitrate, Leunasalpeter, ammonium sulfate, urea, the mixture of organic and ammoniacal nitrogen, and ammonium phosphate all seem to be almost equally good from the weights of dry matter produced.

As yields do not tell the complete story of the availability of nitrogenous compounds the plants were analyzed for total nitrogen and the amounts of nitrogen assimilated from the various sources calculated. The results are given in table 2.

The results are a little different than those from the total yields of dry matter. Rice growing on the ammonium sulfate treatment assimilated the most nitrogen. However, the amounts taken up from the sodium nitrate, ammonium phosphate, Leunasalpeter, and cotton seed meal mixture are almost as great as that from the ammonium sulfate. On the basis of amounts assimilated they could all be considered as good fertilizers for rice.

The results of the analyses of the plants from the cotton seed meal and blood meal treatments show the blood meal to be more available although the total dry weights show them to be of about equal value. The poor results obtained in both experiments with calcium nitrate are rather puzzling. Five different trials have been made in various experiments to get rice to grow when the nitrogen was supplied as calcium nitrate but in all cases the growth has been rather poor. In one experiment the failure to grow was due to a rapid decrease of the pH to alkalinity. It may be possible in the experiments with calcium nitrate when the reaction was fairly well controlled that the chlorosis observed may have resulted from an antagonistic action from the soluble calcium and may have decreased the yield in a manner similar to that reported by Willis and Carrero (25) for calcareous soils.

The relative availability of the different compounds, ammonium sulfate being used as 100, are given in table 3.

The relative availabilities for the separate experiments with the exception of calcium cyanamid agree very well. The averages for the two experiments show that ammonium sulfate, Leunasalpeter, urea, sodium nitrate, blood meal, and a mixture of cotton seed meal and ammonium sulfate should produce good yields of rice if no other factors affect the ability of the plant to utilize the nitrogen or affect the availability of the nitrogen in the soil.

NITROGEN CHANGES

The changes taking place in nitrogenous compounds under anaerobic conditions may depend to some extent upon the form which is present when the anaerobic condition develops. It is generally understood however, that the loss of some total nitrogen as well as a great decrease in available nitrogen may take place when anaerobic conditions develop. Greaves (9) states that the loss of nitrogen by denitrification may occur from the breaking down of complex proteins into simpler products from the reduction of nitrates to ammonia or free nitrogen, and from the transformation of nitrates or ammonia to more complex proteins.

In order to study the effect of flooding upon nitrogenous fertilizers, 300 mgm. of nitrogen was added to 12 kgm. of quartz sand to which was applied a nitrogen-free nutrient solution and 100 cc. of a water extract made from a field soil

which had been cropped to rice. The moisture content was maintained as near 15 per cent as possible for five weeks by the addition of distilled water. At this time the cultures were flooded with distilled water so that water stood one inch deep on the surface of the sand. The water was maintained near this level until the end of the experiment by the daily addition of distilled water. The reaction of the cultures was maintained as near pH 6.5 as possible by the semi-weekly addition of dilute hydrochloric acid or sodium hydroxide as necessary until a period of two weeks, constancy of the pH made further additions unnecessary.

After irrigation, samples were taken every week to determine the total amounts of water-soluble nitrogen present as ammonia, nitrites, and nitrates. In future discussion the phrase "water-soluble nitrogen" will imply those three forms. The samples were taken in the following manner: the cultures were stirred thoroughly and left standing for 24 hours to let all solubility reactions come as near to equilibrium as possible. Then samples of liquid, equivalent to one-fortieth of the original amount of nitrogen, were taken by weighing the jars and taking the required amount from the supernatant liquid. Although objections may be raised as to the accuracy of this method of sampling, preliminary experiments had shown that it was practically impossible to get similar aliquots of the sand and water. For this reason aliquots taken as described probably more nearly represented the amount of soluble nitrogen present than could have been obtained by trying to get the proper mixture of sand and liquid.

The samples were made to 250 cc. with distilled water and thoroughly mixed. Duplicate aliquots were taken for determination of nitrate, nitrite, and ammoniacal nitrogen. The method used for analysis for nitrate and nitrite nitrogen have been reported in a previous paper (1). The ammonia was determined by distilling 50-cc. aliquots with sodium carbonate and catching the distillate in 0.01 *N* HCl. The distillate was made to a volume of 200 cc. with distilled water, aliquots were taken and treated with Nessler's reagent. The amount of nitrogen present as ammonia was determined colorimetrically by comparison with standard solutions which had been treated with Nessler's reagent.

In order to determine the amounts of nitrogen lost by denitrification processes the free water was permitted to evaporate from the uncropped jars nine weeks after irrigation. The jars were weighed and the sand was thoroughly mixed in a galvanized iron tub. Samples were taken for total nitrogen analyses. The analyses were made in triplicate using the salicylic acid modification to include nitrogen in all forms. The ammonia in the distillate was determined by the use of Nessler's reagent.

For ease of study the compounds have been grouped according to the form of nitrogen they contained.

Nitrate nitrogen

The results of duplicate analyses of the changes taking place in nitrogen under anaerobic conditions are given in table 4.

A study of the results gives some very interesting information. The loss of nitrogen from nitrates regardless of whether it is being used as a source of oxygen for bacteria or whether it is being reduced to ammonia is generally regarded as taking place in several stages, during one of which nitrites are supposed to be found. The data reveal that only in one analysis of samples from the sodium nitrate treatment was any appreciable amount of nitrite nitrogen found and only very small amounts were found in the samples from the calcium nitrate treatment. The latter statement is particularly interesting when we consider the amounts of nitrogen as ammonia evidently reduced from nitrates found on several occasions. A possible explanation may be that the reduction of nitrites to ammonia may take place just as rapidly as has been shown (1) for the change from nitrites to nitrates. If such were the case only during certain periods of activity, similar to those suggested by Miyake (7)

TABLE 4
Effect of anaerobic conditions on nitrate nitrogen

| WEEKS AFTER PLANTING | WEEKS AFTER IRRIGATION | N IN NaNO_3 CHANGED TO | | | N IN $\text{Ca}(\text{NO}_3)_2$ CHANGED TO | | |
|----------------------|------------------------|---------------------------------|---------------|---------------|--|---------------|---------------|
| | | NH_3 | NO_2 | NO_3 | NH_3 | NO_2 | NO_3 |
| | | <i>mgm.</i> | <i>mgm.</i> | <i>mgm.</i> | <i>mgm.</i> | <i>mgm.</i> | <i>mgm.</i> |
| 5 | 0 | None | Trace | 84.4 | None | Trace | 26.4 |
| 6 | 1 | None | Trace | 33.6 | 16.0 | Trace | 24.8 |
| 7 | 2 | None | Trace | 83.6 | 37.2 | Trace | 62.4 |
| 8 | 3 | None | 0.28 | 127.0 | 3.6 | Trace | 86.8 |
| 9 | 4 | None | Trace | 111.2 | 4.4 | Trace | 44.1 |
| 10 | 5 | 8.4 | 4.50 | 97.2 | 24.0 | 0.80 | 90.0 |
| 11 | 6 | None | 0.20 | 93.5 | None | 0.40 | 31.2 |
| 14 | 9 | Trace | 14.84 | 20.0 | None | None | Trace |

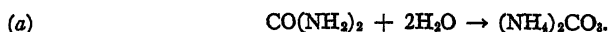
for aerobic conditions, would nitrites be present and the finding of large amounts of nitrite nitrogen in the samples would be due to the accidental sampling of the jars during a period of great activity.

It seems very improbable from the results of these experiments that the reduction of nitrate to nitrite in sufficient quantities to be toxic to plants takes place as suggested by Kelley (14) and cannot account for the failures reported (6, 14, 18) of sodium nitrate to produce as good yields as ammonium sulfate. This agrees with results presented by Janssen and Metzger (13) who found only very small quantities of nitrites present, under irrigated conditions, from applications of sodium nitrate to a rice soil.

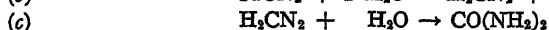
At no time was there much over one-third of the 300 mgm. of nitrogen originally added in a soluble form. This would suggest that loss had been due either to rapid denitrification or to the assimilation of a large amount by bacteria. That the latter action is an important cause of the decrease in soluble nitrogen will be shown later.

Ammoniacal- and ammonia-producing nitrogen

Ammonia-producing compounds are those such as urea and calcium cyanamid which upon coming into contact with water hydrolyze to form ammonia. Urea decomposes according to the following formula,



Cyanamid hydrolyzes first into urea according to these reactions,



and then from urea into ammonia according to the formula (a) given for the hydrolysis of urea. The changes in the soil after hydrolysis had taken place would be the same as for those fertilizers applied directly as ammonium salts. The changes taking place from the nitrogen in these compounds under anaerobic conditions may be studied from the results given in table 5.

With the exception of two samplings of the ammonium sulfate treatment the water-soluble nitrogen was never during the experiment over one-third of that originally added. In many cases the water-soluble nitrogen was only a few per cent of that originally added. Although it might be surmised that some of the nitrogen had been lost by denitrification processes it is also probable that some was converted to an insoluble form, by assimilation by bacteria and by adsorption by the sand as suggested by Wolkoff (26).

The results of the analyses from the urea treatment are rather interesting. Starting with a trace of nitrate nitrogen at the time of irrigation there was an increase in the nitrates present for three weeks and then a decrease until only a trace remained. There was a large increase in all three forms of nitrogen at the third sampling of the urea treatment, whereas in the samples taken a week later there was an increase in nitrate nitrogen only and a decrease in the other two forms. Whether the nitrites present were the intermediate stage of nitrification made possible by oxygen stirred into the cultures in preparation for the sampling can only be a matter of speculation, for it can not be proved from the data presented and, as far as is known, there have been no data pertaining to similar conditions presented in the literature. The data from the seventh sampling of the calcium cyanamid treatments also indicate that some oxidation of ammonia is taking place, because there is a decided increase in nitrite nitrogen accompanied by a large decrease in ammoniacal nitrogen.

In only one sample, the third sampling from the urea treatment, was there any large amount of nitrites present. This seems rather remarkable when such quantities of nitrates disappeared in a week or two from different treatments. Such a rapid loss of nitrate nitrogen without the appearance of nitrite nitrogen tends to substantiate the contention previously made that the nitrate is reduced so rapidly that the finding of large quantities present at any one time may be due to the accidental sampling during a cycle of activity of nitrate-reducing bacteria.

TABLE 5
Effect of anaerobic conditions on ammoniacal-nitrogen- and ammonia-producing compounds

| WEEKS AFTER PLANTING | WEEKS AFTER IRRIGATION | N IN $(\text{NH}_4)_2\text{SO}_4$ CHANGED TO | | | N IN $(\text{NH}_4)_3\text{PO}_4$ CHANGED TO | | | N IN UREA CHANGED TO | | | N IN CALCIUM CYANAMID CHANGED TO | | |
|-------------------------|---------------------------|--|---------------|---------------|--|---------------|---------------|----------------------|---------------|---------------|-------------------------------------|---------------|---------------|
| | | NH_3 | NO_2 | NO_3 | NH_3 | NO_2 | NO_3 | NH_3 | NO_2 | NO_3 | NH_3 | NO_2 | NO_3 |
| | | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| 5 | 0 | 68.0 | Trace | Trace | 45.6 | Trace | Trace | 62.4 | 1.92 | Trace | 22.0 | Trace | Trace |
| 6 | 1 | 44.4 | Trace | Trace | 58.8 | Trace | Trace | 14.4 | 0.78 | 9.0 | 34.0 | Trace | Trace |
| 7 | 2 | 177.2 | 0.24 | Trace | 49.2 | 0.16 | Trace | 67.0 | 40.0 | 31.2 | 1.6 | Trace | 3.3 |
| 8 | 3 | 202.0 | 0.12 | Trace | 51.6 | 0.68 | Trace | 42.8 | Trace | 41.6 | Trace | Trace | Trace |
| 9 | 4 | 92.0 | None | Trace | Trace | 0.43 | Trace | 2.4 | Trace | 11.8 | Trace | Trace | Trace |
| 10 | 5 | 104.0 | Trace | Trace | Trace | Trace | Trace | 4.0 | 0.64 | 9.0 | 32.0 | Trace | Trace |
| 11 | 6 | 67.2 | None | Trace | Trace | Trace | Trace | Trace | 0.24 | Trace | Trace | 7.16 | Trace |
| 14 | 9 | Trace | None | Trace | Trace | None | Trace | Trace | None | Trace | Trace | None | Trace |

Organic nitrogen

The transformations taking place in organic nitrogen under anaerobic conditions would be radically different from those described for the preceding processes because the nitrogen is in a complex form and would have to be liberated in a simpler form before it would be available to plants. It is true that because several weeks elapsed before irrigation some of the nitrogen could

TABLE 6
Effect of anaerobic conditions on organic nitrogen

| WEEKS AFTER PLANTING | WEEKS AFTER IRRIGATION | N IN COTTON SEED MEAL CHANGED TO | | | N IN BLOOD MEAL CHANGED TO | | |
|----------------------|------------------------|----------------------------------|-----------------|-----------------|----------------------------|-----------------|-----------------|
| | | NH ₃ | NO ₂ | NO ₃ | NH ₃ | NO ₂ | NO ₃ |
| | | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| 5 | 0 | 67.2 | 4.76 | Trace | 28.0 | 2.32 | Trace |
| 6 | 1 | 4.0 | 0.26 | Trace | 8.4 | 0.68 | Trace |
| 7 | 2 | 49.2 | 0.06 | 8.9 | 49.2 | 0.13 | Trace |
| 8 | 3 | 39.6 | 0.16 | 28.4 | 54.4 | 0.15 | Trace |
| 9 | 4 | 8.6 | 0.30 | Trace | 7.6 | Trace | Trace |
| 10 | 5 | 14.0 | 3.88 | Trace | 4.40 | 0.28 | Trace |
| 11 | 6 | Trace | 0.35 | Trace | Trace | 0.32 | Trace |
| 14 | 9 | Trace | None | Trace | Trace | None | Trace |

TABLE 7
Effect of anaerobic conditions on mixtures of nitrate and organic and ammoniacal nitrogen

| WEEKS AFTER PLANTING | WEEKS AFTER IRRIGATION | N IN LEUNASALPETER CHANGED TO | | | N IN COTTON SEED MEAL AND NaNO ₃ CHANGED TO | | | N IN COTTON SEED MEAL AND (NH ₄) ₂ SO ₄ CHANGED TO | | |
|----------------------|------------------------|-------------------------------|-----------------|-----------------|--|-----------------|-----------------|--|-----------------|-----------------|
| | | NH ₃ | NO ₂ | NO ₃ | NH ₃ | NO ₂ | NO ₃ | NH ₃ | NO ₂ | NO ₃ |
| | | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| 5 | 0 | 88.4 | Trace | 20.0 | 6.8 | 2.88 | 19.4 | 29.2 | 3.52 | Trace |
| 6 | 1 | 10.8 | Trace | 17.8 | Trace | 3.12 | 24.2 | 16.0 | 0.50 | Trace |
| 7 | 2 | 94.8 | 0.12 | 35.7 | Trace | 16.95 | 55.6 | 127.2 | 0.04 | Trace |
| 8 | 3 | 99.6 | Trace | 66.5 | Trace | 0.40 | 91.0 | 140.4 | 0.28 | Trace |
| 9 | 4 | 48.8 | Trace | 25.3 | Trace | 0.90 | 50.0 | 33.4 | Trace | Trace |
| 10 | 5 | 30.8 | 0.64 | Trace | Trace | 1.60 | 6.4 | 30.6 | Trace | Trace |
| 11 | 6 | 12.8 | Trace | Trace | Trace | 3.71 | 14.7 | Trace | Trace | Trace |
| 14 | 9 | Trace | None | Trace | Trace | None | Trace | Trace | Trace | Trace |

have been converted to more available forms than contained in the organic form. The changes taking place can be explained from the analyses in table 6.

For the major part of the experiment, nitrogen as ammonia was being liberated from the organic nitrogen. In connection with this the small concentrations of nitrite nitrogen present in nearly all samplings, because they are more consistent than in almost any other treatment, suggest that the changes of organic nitrogen may have been carried farther than the ammonia form. The

data for the samples taken the second and third week after flooding are suggestive of nitrification reactions and agree with suggestions previously made. As would be expected from the form of nitrogen used, there were never any large amounts of water-soluble nitrogen present in the cultures.

Mixed forms of nitrogen

Each form of nitrogen in a mixture of nitrogenous fertilizers might be expected to behave as though present alone. That such is the case may be seen by the results presented in table 7.

The results agree on the whole with those of the other experiments in that at no period of sampling was there much over one-third of the nitrogen present in a soluble form. The amounts of nitrite nitrogen found at the different periods of sampling were also small.

TABLE 8
Losses of nitrogen from various nitrogenous fertilizers in anaerobic sand cultures

| SOURCE OF NITROGEN | N ADDED | FOUND BY ANALYSES | LOST |
|--|-------------|-------------------|-----------------|
| | <i>mgm.</i> | <i>mgm.</i> | <i>per cent</i> |
| NaNO ₃ | 295.0 | 95.2 | 67.8 |
| CaNO ₃ | 302.0 | 165.2 | 45.3 |
| (NH ₄) ₂ SO ₄ | 302.8 | 223.2 | 26.2 |
| (NH ₄) ₃ PO ₄ | 152.6 | 135.2 | 11.4 |
| Urea..... | 264.0 | 143.8 | 45.5 |
| Calcium cyanamid..... | 300.0 | 120.8 | 59.8 |
| Cotton seed meal..... | 305.5 | 88.8 | 71.0 |
| Blood meal..... | 300.0 | 137.5 | 54.2 |
| Leunasalpeter..... | 296.5 | 123.2 | 58.4 |
| Cotton seed meal and NaNO ₃ | 300.2 | 76.0 | 74.7 |
| Cotton seed meal and (NH ₄) ₂ SO ₄ | 304.1 | 205.6 | 32.4 |

Losses of nitrogen by denitrification

Losses of nitrogen by denitrification from rice soils might greatly decrease the yields of rice. From a theoretical consideration (23) nitrogen from nitrates would be lost in the greatest amounts. This does not preclude that possibility that losses from organic and ammoniacal forms may be just as great as from nitrate nitrogen. The results in table 8 were secured from an experiment to determine the amounts lost from the various compounds which could be used for nitrogenous fertilizers.

The most striking result is the fact that considerable amounts of nitrogen were lost from all sources except the strict ammoniates. The losses from nitrates were larger than those from the true ammoniates, such as ammonium sulfate and ammonium phosphate, but on the other hand the ammonia-producing compounds and the organic nitrogenous compounds lost as much as or in some cases more than the nitrates. Although some of the losses from the compounds

may be attributed to loss from nitrates formed by nitrification processes before irrigation, tables 4 to 7 show that in some instances practically no nitrate nitrogen was produced. Ammonium sulfate is a very good example to use. There is shown a total loss of 26.0 per cent and yet at no time was there more than a trace of nitrates present. Blood meal, calcium cyanamid, and the mixture of cotton seed meal and ammonium sulfate acted in a similar way, showing a loss of 54.0, 59.8, and 32.4 per cent respectively of the nitrogen added and yet there was never more than a trace of nitrates present during the entire experiment.

The results do not show that denitrification of nitrates with the production of nitrites, as suggested by Kelley (14), is the chief cause of the failure to produce good crops of rice. In only one case, a late sampling of sodium nitrate treatment in a series of experiments mentioned in the following, did the nitrite concentration approach that which he considers toxic. In most cases the concentration was much less than 1 p.p.m. and according to Perciabosco and Rosso (19) was dilute enough for direct assimilation by rice.

The losses occurring from the organic nitrogenous compounds were probably due to the anaerobic decomposition of the compound, a process similar to that taking place in paddy soils, as reported by Harrison and Aiyer (11). They state that the gaseous nitrogen found in paddy soils arises from the decomposition of the organic matter, green manure, and decomposing roots in the soil.

A second series of experiments were made but because of the higher temperature at which they were run and the shorter duration of the experiments they could not be compared directly with the results reported above. However, the same conclusions may be drawn from the results as from the above experiments; viz., (a) At no time was much over one-third of the nitrogen originally added present in a soluble form; many times only a few per cent was present in a soluble form. (b) Nitrites were only found in small amounts in the different treatments, including the nitrate nitrogen fertilizers. (c) Nitrogen was lost by denitrification processes from all the different forms of nitrogen, but the losses from the strict ammoniates such as ammonium sulfate and ammonium phosphate, were somewhat smaller than from all the other forms.

DISCUSSION OF RESULTS

Nitrogen whether in nitrate, organic, or ammonia form seems to be readily available for rice if other growth factors such as reaction, temperature, and light are maintained uniform for all treatments. There may be some differences in certain organic compounds due to differences in the rate at which they decompose and liberate nitrogen. Although no attempt will be made to discuss the results from the standpoint of explaining each conflicting experiment mentioned in the literature review, certain facts which appear to be fundamental will be discussed from the data presented. It has been suggested (14) that nitrates should not be used for rice because it would not produce good yields.

The evidence presented shows that when the reaction is maintained constant, nitrogen from sodium nitrate is assimilated almost as readily as from ammonium sulfate. There is a possibility that part of the nitrate nitrogen may be reduced to ammonia or it may be assimilated by bacteria and later liberated from their bodies in the ammonium form and utilized as such by the plants.

When no precautions are taken to prevent the media from becoming more alkaline there is a decidedly injurious effect from the use of sodium nitrate for fertilization of rice, as has been shown previously (2). The use of sodium nitrate, or in fact any physiologically basic fertilizer, on a slightly acid to neutral soil may result in no response or even a decreased yield from the fertilizer. On the other hand nitrates could probably be used on distinctly acid soils to good advantage.

The reduction of nitrates to free nitrogen did not seem to take place any faster from nitrates than from any other compound, exclusive of ammonium sulfate and ammonium phosphate, so that only under very limited conditions might the amount of nitrogen lost from nitrates by denitrification seriously affect the crop yield. The loss after four months in uncropped cultures was 67.8 per cent of the nitrogen added. As a matter of fact, if the jars had been cropped the loss would probably not have been nearly so great, for analyses of the crops showed that they assimilated 63.6 per cent of the nitrogen added as sodium nitrate. This would give a loss of 36.4 per cent by denitrification, assuming that all the nitrogen not taken up by the plant was denitrified, which was probably not the case. Part of the 36.4 per cent had probably been assimilated by bacteria and retained in their bodies.

The organic compounds, if they are readily decomposed, are well adapted for the fertilization of rice. However, there appears to be as much danger of losing nitrogen from them as from sodium nitrate. Having practically no effect on the soil reaction, they could probably be used under practically all soil conditions. If considerable time elapses from their application to their irrigation nitrification may take place and the situation would be somewhat similar to that where sodium nitrate was applied. A similar condition would be produced from the plowing under of crop residues and green manures.

Ammonium fertilizers seem best adapted for all conditions. Although they may produce a considerable increase in the pH, the rice plants seem to be able to stand rather large changes without showing much injury. The compounds could be used on most soils regardless of reaction. Also they do not seem to lose as much nitrogen by denitrification process as the other compounds. Part of this may be due to the fact that ammonia may be adsorbed by soil complexes and is not available in as large quantities for biological activities as are some of the other compounds.

Why so much more nitrogen should be lost from fertilizers such as urea and cyanamid, which produce ammonium carbonate by hydrolysis, than from the strict ammoniates can not be readily explained. Part of it may have been due to a denitrification process similar to that taking place from organic matter.

Growth with Leunasalpetar, in which the nitrogen is three-fourths ammonia-

cal and one-fourth nitrate, was within 4 per cent as effective in producing rice as was ammonium sulfate when ammonium sulfate was taken as 100. This seems to indicate that any form of nitrogen as long as it changed either to the nitrate or ammonium form would be readily assimilated by rice.

Mention has been previously made that all the rice became rather chlorotic shortly after flooding. This condition was very similar to that observed under field conditions even when nitrogenous fertilizers are applied. The results of the nitrogen transformation studies suggest that the chlorotic condition was due to an insufficient supply of available nitrogen.

SUMMARY

The availability of the various forms of nitrogen was determined by plant growth and chemical analysis of the plant tissue to determine the amount of nitrogen assimilated. Studies were made of the transformations taking place in various nitrogenous compounds under anaerobic conditions to determine whether these changes might affect the suitability of these compounds for the nutrition of rice. A brief summary of the results follows.

When proper precautions were taken the efficiency of the following nitrogenous compounds, compared to ammonium sulfate as 100 were, Leunasalpeter 96 per cent, a mixture of cotton seed meal and ammonium sulfate 96 per cent, urea 92 per cent, sodium nitrate 89.5 per cent, blood meal 87 per cent, ammonium phosphate 84.5 per cent, calcium cyanamid 69.5 per cent, a mixture of cotton seed meal and sodium nitrate 66 per cent, cotton seed meal 61.5 per cent, and calcium nitrate 59 per cent. Under proper conditions the first six seem well adapted for the production of rice. However, it is safer to recommend the ammonium compounds, such as ammonium sulfate and ammonium phosphate, because rice seems to be affected less by the changes they produce and there is the probability that less nitrogen may be lost by denitrification.

Organic forms of nitrogen may be recommended highly because they produce good results and have practically no effect on the soil reaction. There is danger, however, of loss of considerable amounts of nitrogen through denitrification. Although sodium nitrate gave good results under controlled conditions its general use should not be recommended because of the sensitiveness of rice to decreases in pH. The same thing would apply for all physiologically basic fertilizers.

The production of nitrites due to denitrification was very spasmodic and only in several cases were they found in quantities larger than several mgm. per jar. Nitrites were found in jars which had received no nitrate nitrogen. The results do not support the contention that the production of nitrites from nitrates is the cause for the failure of nitrates to produce good yields of rice.

Nitrogen was lost, presumably as elemental nitrogen, from all form of nitrogen whether it was in the ammonium, nitrate, or organic form, but smaller amounts were lost from the ammonium compounds, such as ammonium sulfate and ammonium phosphate, than from nitrates and organic nitrogen. Denitrification of nitrates was no greater than that of organic compounds such as

blood meal, cotton seed meal, and urea. The denitrification process took place so rapidly that only small quantities of nitrogen as nitrites were found from most treatments.

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SOIL TYPE AND CROP ADAPTATION

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That there is a vast difference in the response of the same crops to different soil types in Florida is common knowledge. In fact, if this is not given due consideration, trucking and fruit growing in portions of the state are more or less hazardous. With the knowledge that this condition does exist and with the realization of the value of a practical working knowledge of it in connection with successful farm operations, an attempt has been made to collect as much detailed information as possible to bring out the relationship between soil type and crop adaptation at Penny Farms.

EXPERIMENTAL

Nine of the most prominent soil types occurring at Penny Farms have been used in connection with this work. They are Norfolk fine sand flat phase, Norfolk fine sand, Leon fine sand, Leon fine sand loamy phase, Blanton fine sand, St. John's loamy fine sand, Portsmouth loamy fine sand, Portsmouth fine sand, and Portsmouth fine sandy loam. These soils vary widely in their general characteristics and crop-producing power. The surface of the Norfolk fine sand, to a depth of 5 to 6 inches, is light gray, incoherent fine sand. The subsoil, to a depth of 36 inches or more, is a yellowish gray to pale yellow, loose fine sand. It is deficient in humus and is considered to be very poor for general truck crops. The Norfolk fine sand, flat phase, consists of a gray to dark gray, incoherent fine sand, underlain at about 8 to 10 inches by a yellow, pale yellow, or grayish yellow incoherent fine sand, extending to a depth of more than 3 feet. This type is somewhat superior to the Norfolk fine sand. The Leon fine sand consists of a gray to whitish gray, loose and incoherent fine sand, which at from 15 to 34 inches is underlain by a reddish brown to dark brown (and sometimes black) compact sand or organic hardpan layer. The immediate surface for 2 to 4 inches may be a gray or dark gray fine sand, the dark coloring being organic coloring from decayed grass roots. The hardpan layer ranges from a few inches to 2 or 3 feet in thickness. Usually it is from 6 to 14 inches in thickness. Below the hardpan layer is a moist, loose, incoherent fine sand having the nature of quick sand, when saturated. The Leon fine sand, loamy phase, is a gray to dark gray fine sand, 1 to 5 inches deep, overlying a light gray to almost white, rather incoherent fine sand which at

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depths varying from 8 to 30 inches, though usually at about 15 to 22 inches, passes into a dark brown or rusty brown and sometimes black, dense hardpan layer. This rages from 3 inches to 2 feet in thickness, and is underlain by a white, fine sand which is always moist and compact, but when disturbed becomes incoherent and has the nature of quicksand. Blanton fine sand consists of 2 to 4 inches of a gray to dark gray fine sand underlain by a yellowish gray to grayish white fine sand 25 to 40 inches thick, resting upon a very light grayish yellow to light gray fine sand.

Portsmouth loamy fine sand is a very dark brown to black loamy fine sand containing a high percentage of organic matter. This dark organic material extends to 15 to 24 inches then changes to a dark gray to gray fine sand, gradually becoming lighter with depth. Portsmouth fine sandy loam is identical with the Portsmouth loamy fine sand, except that it is slightly higher in organic matter than the latter and has a sticky gray sandy clay loam in the lower subsoil at 34 to 36 inches. Portsmouth fine sand differs mainly from Portsmouth loamy fine sand in that it has a layer of white sand from 15 to 25 inches below the surface. St. John's loamy fine sand, to a depth of 5 to 10 inches, consists of a very dark gray to black loamy fine sand. Below this is a light gray to grayish white, incoherent fine sand which is underlain at depths ranging from 10 to 36 inches, but ordinarily 22 to 28 inches, by a dark brown to black, compact, organic hardpan. The layer of hardpan, which ranges from 2 to 6 inches in thickness, rests upon a light gray, incoherent fine sand.

For convenience the two Norfolk types are grouped as Norfolk and the three Portsmouth types as Portsmouth and are treated as such in the remainder to this article.

In connection with the development of the farm operations of the J. C. Penny-Gwinn Corporation at Penny Farm, Florida, it was deemed necessary that a detailed soil survey on the scale of 2 inches to the mile be made of the entire tract and that a careful study be made of the response to different soil types of all crops grown. In keeping with this plan some very complete and extensive data have been collected to show that there is a striking relationship between soil type and crop adaptation at Penny Farms.

For this study Irish potatoes, grapes, peaches, pears, pecans, plums, blueberries, tung oil, crotalaria, corn, pepper, Japanese sugar cane, peppermint, sweet potatoes, and numerous other crops are used. These crops will be discussed in the order mentioned.

All data reported here for Irish potatoes were obtained from fields where the soil survey showed the soil to be uniformly of one type throughout. All acreages in connection with the potato crop were accurately chained, and a complete set of field notes kept on each field used throughout the season. All yield data represent accurately measured yields. The data were all obtained in connection with actual field operations.

Some 400 acres or more were planted to potatoes between January 20 and February 10, 1927, on the leading soil types mentioned, for the spring crop.

Uniform fertilizer treatment and cultural practices were used on the entire acreage, thus making it possible to obtain comprehensive data bearing on the relation of soil type to potato production during the season.

The data contained in table 1 were obtained by taking the average yield of no. 1 potatoes for a few of the representative fields located on Norfolk soil as 100 per cent and similar averages for each of the other soil types and expressing these in per cent. An examination of the data clearly reveals a decided relationship between soil type and potato production with the spring crop. The heavier St. John's and Portsmouth soils proved to be far superior to the lighter, drier Norfolk, Leon fine sand loamy phase, and Blanton soils.

TABLE 1
Relation of soil type to potato yield in the spring of 1927

| SOIL TYPE | YIELD ON PERCENT- AGE BASIS |
|----------------------------------|--------------------------------|
| Norfolk..... | 100 |
| Leon fine sand, loamy phase..... | 170 |
| Blanton fine sand..... | 171 |
| St. John's loamy fine sand..... | 224 |
| Portsmouth..... | 264 |

TABLE 2
Relation of soil type to potato yield in the fall of 1927

| SOIL TYPE | YIELD EXPRESSED ON PERCENTAGE BASIS |
|----------------------------------|--|
| Norfolk..... | 100 |
| Leon fine sand, loamy phase..... | 140 |
| Blanton fine sand..... | 155 |
| St. John's loamy fine sand..... | 305 |
| Portsmouth..... | 321 |

The Norfolk soils made the poorest showing of all the types used. They proved to be very unsatisfactory for potatoes in every case. This is largely because of their lack of humus, their open, sandy character, and their inability to retain moisture during the growing season.

The Blanton fine sandy soil, which is somewhat heavier, slightly more compact, and less droughty than the Norfolk soils, proved to be a superior potato soil to the latter. This soil, however, did not rate as a first-class potato soil, as may readily be seen. It, too, is poor at retaining moisture.

The loamy phase of Leon fine sand proved to be only a medium potato-producing soil. Because of the peculiar nature of this soil, it, too, has a tendency to be droughty or very poor at retaining moisture.

The St. John's loamy fine sand, which is much heavier and more compact than either of the aforementioned soils, was superior to them in producing

potatoes. The high organic matter content, its compact nature, and its natural low position make it very efficient at holding moisture, which accounts in part for its superiority as a potato soil.

The Portsmouth soils easily proved their superiority for potato production over all the other types during this period. This is largely because of their larger humus content, more compact nature, and ability to retain moisture during drought.

Potatoes were planted on the same types of soil during the fall of 1927, but on a much smaller acreage, with the results listed in table 2.

The figures in table 2 were obtained by taking the average total acre yield of graded potatoes for all the Norfolk acreage planted as 100 per cent and similar averages for each of the other soil types and expressing these in per cent.

TABLE 3
Soil type and potato yields for the spring of 1928

| SOIL TYPE | NUMBER OF ACRES PLANTED | TOTAL YIELD IN BARRELS | AVERAGE ACRE YIELD IN BARRELS | YIELD EX- RESSED ON PERCENTAGE BASIS |
|------------------------------------|-------------------------------|---------------------------|-------------------------------------|---|
| Norfolk fine sand, flat phase..... | 16.10 | 919 | 57.0 | 100 |
| Leon fine sand, loamy phase..... | 11.20 | 566 | 50.7 | 89 |
| Blanton fine sand..... | 80.05 | 5,091 | 63.7 | 112 |
| St. John's loamy fine sand..... | 5.00 | 293 | 58.6 | 103 |
| Portsmouth..... | 132.20 | 8,534 | 64.5 | 113 |

TABLE 4
Average monthly rainfall in inches from 1876 to 1926

| | |
|-------------------|--------------------|
| January.....2.75 | July.....6.50 |
| February.....3.75 | August.....6.30 |
| March.....3.90 | September.....5.30 |
| April.....3.30 | October.....3.75 |
| May.....5.00 | November.....1.90 |
| June.....6.75 | December.....3.60 |

The percentage figures for the various soil types of the fall crop are not exactly the same as the corresponding figures for the spring crop, to be sure, but there is indeed a very close correlation between them. In every instance, the various soil types for the fall crop maintained the same positions in the scale of relative values as for the spring crop. This shows conclusively that there was a decided relationship between soil type and potato production during the spring and fall of 1927.

The results reported above were obtained during seasons when the rainfall was about average or normal for this section. Since moisture is often the limiting or controlling factor in potato production on these soils, it is natural to expect those soils of low water-holding capacity to suffer more severely than

those capable of retaining large quantities of moisture during normal or dry seasons. However, with the moisture factor satisfactorily solved, either by means of an adequate amount of rainfall properly distributed through the growing season or by irrigation, this relationship will be much less noticeable. On the other hand, should there be a surplus rainfall the heavy soils, having a

TABLE 5
Daily rainfall in inches for first six months of 1928

| DAY | JANUARY | FEBRUARY | MARCH | APRIL | MAY | JUNE |
|-----------|---------|----------|-------|-------|------|------|
| 1 | 0.08 | | | | | |
| 2 | | | | | | 0.25 |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | 0.95 | 0.06 |
| 8 | | | | | 0.08 | |
| 9 | 0.07 | | | | | |
| 10 | | | | 1.40 | | 1.15 |
| 11 | | | 0.52 | 0.91 | | |
| 12 | | | | | | |
| 13 | | 0.42 | | | 0.05 | |
| 14 | | | | | | 0.08 |
| 15 | | | | 3.60 | | 2.50 |
| 16 | | 0.33 | 0.06 | | | 1.40 |
| 17 | | | 0.70 | | | |
| 18 | | 0.47 | 0.07 | | | |
| 19 | | | | | | |
| 20 | 0.04 | | | | | |
| 21 | | | | | 0.19 | 0.20 |
| 22 | 0.22 | | | 0.60 | 0.40 | 0.90 |
| 23 | | 0.04 | | 1.25 | 0.84 | 0.22 |
| 24 | | 1.50 | | | | 0.20 |
| 25 | | 0.12 | 0.20 | | | 0.85 |
| 26 | | | 1.20 | | | 0.25 |
| 27 | 0.30 | | 0.10 | 2.30 | | |
| 28 | | | | | | 0.40 |
| 29 | | | | | | |
| 30 | | | 0.12 | | 1.65 | |
| 31 | | | | | 0.25 | |
| Total.... | 0.71 | 2.88 | 2.97 | 10.06 | 4.41 | 9.18 |

natural low position and an extremely high water-holding capacity, will be much more easily affected adversely than the lighter and more porous ones. This is clearly borne out by the data given in table 3 which were obtained from the 1928 spring potato crop.

In preparing the data in table 3, only the results from those fields where the soil was uniformly of one type were used. There is a wide variation in the total

acreages listed for the different soil types which naturally will affect the final results to some extent, but which may enable one to get some idea of the differences in the response of some of the soil types to potato production during the spring of 1928 as compared with that of the spring and fall of 1927.

It is indeed interesting to note that the Norfolk and Blanton soils compared very favorably with the Portsmouth and St. John's loamy fine sand soils as potato soils during this season and were superior to Leon fine sand, loamy phase. On first thought it may appear difficult to explain this peculiarity in light of the results reported in tables 1 and 2. However, an examination of the rainfall data in tables 4 and 5 will reveal the proper explanation.

Table 4 shows the average monthly rainfall in inches for this area from 1876 to 1926. The average rainfall for January, February, March, and April, the spring potato season, is very light. The same is true for October, November, and December, the fall potato season. The rainfall for 1927 was about average.

The figures in table 5, however, reveal a different situation. The rainfall during the spring potato season of 1928 was much above the average.

In view of the fact that the inability of the lighter soils to retain sufficient moisture for maximum potato production during an average season is responsible in large measure for their inferiority as potato soils, the above rainfall data reveal a very satisfactory explanation for the peculiar reversal of form of some of the soil types to potato production during the spring of 1928 over the spring and fall of 1927.

Beginning with the last week in January, the rainfall for the most part was about as near ideal for potato growing as could be expected until the last half of April when the unprecedented sum of 10.06 inches fell from April 10 to 27, inclusive. Until the heavy rainfall occurred during the last half of April the potatoes on the Portsmouth and St. John's soils had a decided lead on those on the lighter, drier types of soil, but even at that the potatoes were doing well on the latter types because of the very favorable rainfall distribution. However, it was during the period of heavy rain in April that the potatoes on the heavy Portsmouth and St. John's soils suffered severely from excessive rainfall, while at the same time the Blanton and Norfolk soils were in excellent condition for optimum potato production. Satisfactory drainage was much more difficult to obtain on the St. John's than on the Portsmouth soils during this period. However, potatoes on both types suffered severely in places. The high water-holding capacity of the St. John's soil, together with its natural low position, made it next to impossible to drain it sufficiently during the heavy rains in April to prevent considerable damage to the crop. During this same period the potatoes on the Blanton and Norfolk soils were being very highly favored by an adequate water supply.

However, in spite of the serious handicap placed on the Portsmouth and St. John's soils by the heavy rainfall in April, the Portsmouth still maintained its lead as a potato-producing soil with St. John's following only slightly behind Blanton, which in turn rated very close to Portsmouth during this season.

The Norfolk soil made a very creditable showing during this period also. In addition to being favored with a satisfactory rainfall distribution, this soil had another decided advantage over the two previous seasons because of the fact that nothing but the flat phase of Norfolk fine sand was planted to potatoes this season, whereas during the two previous seasons considerable Norfolk fine sand was planted. The Norfolk fine sand is much inferior to the flat phase of Norfolk fine sand as a potato-producing soil, and tends to lower the average yield. The flat phase of Norfolk fine sand normally rates very close to Blanton fine sand as a potato soil.

The average acre yield of the Leon fine sand, loamy phase, was somewhat below that of Norfolk fine sand, flat phase, and Blanton fine sand during this season. This is due largely to the fact that areas of the fomer contain pockets of hardpan which prevent water passing through the subsoil fast enough to prevent the plants from being seriously damaged by excessive water during periods of excessive rainfall.

The response of other crops to different soil types is just as pronounced as that with potatoes. This may readily be seen from a study of the discussion and photographs which follow.

Notwithstanding the fact that Blanton fine sand proved to be only a mediocre potato soil it is an excellent soil for grapes. This is clearly borne out by plate 1, figure 1. These plants were set March 4, 1927, and the picture was taken July 21 of the same year. Loamy phase of Leon fine sand had about the same value as a potato-producing soil as Blanton but is far inferior to Blanton as a grape soil. This is clearly brought out by a comparison of figures 1 and 2, plate 1. The grapes shown in figure 2 were set the same time as those in figure 1 and the picture was taken on the same day. The cultural treatments for the two vineyards were as nearly the same as possible. The difference in vine growth is due almost wholly to soil.

Since Blanton fine sand and loamy phase of Leon fine sand had almost equal values as potato soils, at first thought it may seem difficult to explain the difference in their reaction to grapes. However, upon a more careful consideration of the characteristics of these two types of soil as related to the development of the root system of the grape vine one may easily account for this difference. Both of these soils proved to be somewhat droughty, or poor at retaining moisture during an average season, for a first class potato soil. This is due in part, at least, to the fact that the potato crop is shallow rooted and is grown during the season of light rainfall which is not properly distributed to keep the moisture content of these soils right for optimum potato growth. The grape vine has a much more rangy root system than the potato plant and grows through the wet summer season as well as the drier potato season. The nature of the Blanton soil is such that the water table during the period of heavy rainfall in the summer months seldom gets nearer the surface than 4 to 5 feet, whereas with the loamy phase of Leon fine sand it is customary for the water table to rise to within $1\frac{1}{2}$ to 2 feet of the surface of the ground. This means, of course, that

the roots of the grape vine on the Blanton soil have a feeding range down to 4 to 5 feet during the wettest season of the year whereas on the loamy phase of Leon fine sand it can feed only from $1\frac{1}{2}$ to 2 feet deep. Roots penetrating the soil to a greater depth than $1\frac{1}{2}$ to 2 feet on this type of soil during the dry weather are automatically killed by excessive water during the wet summer season. As a result the plants are severely stunted and very often killed outright on the loamy phase of Leon fine sand whereas they are not injured on the Blanton soil.

Blanton soil is also very well adapted to the production of pecans, plums, peaches, and pears as may readily be seen from plate 2, figure 1. The same crops make a complete failure, however, when set on loamy phase of Leon fine sand, Leon fine sand, and St. John's loamy fine sand. This is clearly brought out in plate 2, figure 2. The trees in these two groves were set early in March, 1927, by the same group of men and were treated as nearly alike as was humanly possible. The photographs were taken on July 13 of the same year with the results shown.

Blanton fine sand is a good soil for pecans, plums, peaches and pears for the same reason that it is good for grapes, whereas the loamy phase of Leon fine sand is a poor soil for these crops for the same reason that it is not adapted to the production of grapes.

St. John's loamy fine sand proved to be a very satisfactory soil for potatoes but an extremely poor one for the other crops mentioned. It is a good potato soil largely because it retains moisture well during the potato growing season and is capable of taking care of the moisture needs of the potato crop. On the other hand, it has a water table within from 6 to 9 inches of the surface during the wet summer season, which makes it practically impossible to grow deep rooted crops unless the drainage is such that the water table can be lowered sufficiently to meet their needs. The characteristic low position of this type of soil makes it almost impossible for the water table to be lowered sufficiently to enable these crops to grow.

Blanton fine sand also produces blueberries satisfactorily whereas Leon fine sand makes a complete failure with the same crop. This is clearly brought out in plate 3. The plants were all set out the same time and received similar treatment. The plants in the foreground are on Leon fine sand and those in the background on Blanton fine sand.

Blanton fine sand is a satisfactory soil for blueberries for the same reason it is suited to grapes. Because of its peculiar make-up, the Leon fine sand is decidedly unfit, not only for blueberries but for practically all other crops, unless there is absolute water control practiced. This soil type is characterized by a compact, brownish, organic, hardpan layer ranging from 12 to 18 inches beneath the surface. This hardpan layer is impervious to water and the roots of many cultivated plants, and varies from 1 to several inches in thickness, usually averaging about 3 to 4 inches thick. The soil above it is very coarse and poor at retaining capillary moisture. During periods of low rainfall the small amount of capillary moisture in the soil above the hardpan layer is

quickly exhausted by plants and can not be replenished by capillary movement from below. The plants naturally die from lack of water. On the other hand, during periods of heavy rainfall the surplus water is unable to percolate through this hardpan layer and is forced to accumulate as free water above this layer, thus drowning out whatever crops may have been growing up to that time.

The roots of most cultivated crops are unable to penetrate this peculiar hardpan. However, should they be able to penetrate it they would encounter free water immediately beneath it and further growth would be checked.

The same striking contrast may be had with tung oil trees when planted on Norfolk fine sand and Leon fine sand. This is clearly shown in plate 4, figure 1. At the same time that blueberries, grapes, pecans and tung oil fail on Leon fine sand crotalaria apparently does well. This is shown by plate 4, figure 2.

Norfolk fine sand is somewhat more droughty than Blanton fine sand and also has a lower water table. Ordinarily, however, Norfolk fine sand contains enough moisture during the dry season to meet the requirements of the tung oil tree and has a water table low enough during the wet summer season so as not to interfere with the normal root development of that plant. The tung oil tree fails to make good on Leon fine sand for the same reason that blueberries and most other crops do.

Apparently crotalaria is able to adjust itself successfully to Leon fine sand under some conditions, at least, a fact brought out in plate 4, figure 2. The roots of the crotalaria were able in this case to penetrate the hardpan layer.

Corn is just as sensitive to soil types as any of the other crops mentioned. This is very clearly brought out by plate 5. The corn in figure 2 was planted about one week earlier than that in figure 1. The cultural treatments were practically the same for the two fields. The pictures were taken on July 13, 1927. The corn in figure 1 was planted on Portsmouth loamy fine sand and that in figure 2 on Leon fine sand.

Corn is adapted to Portsmouth loamy fine sand for the same reason that potatoes are adapted to it. It is also unadapted to Leon fine sand for the same reason that most other crops are adapted to it.

Notwithstanding the fact that grapes, pecans, plums, pears, peaches, and many other crops make a complete failure on St. John's loamy fine sand, peppers appear to be perfectly at home on this soil. This is clearly evident from plate 6, figure 1. This soil also rated well as a potato soil. It is equally well adapted to bulbs, onions, peppermint, and numerous other truck crops. On the other hand there are several crops, other than those mentioned, which are wholly unsuited to this soil.

Pepper, bulbs, onions, peppermint, and such crops are well adapted to St. John's loamy fine sand soil for the same reason that potatoes are. However, those crops with a rangy root system are entirely unsuited to this type of soil.

Notwithstanding the fact that Portsmouth loamy fine sand is very poorly adapted to such crops as grapes, peaches, plums, pears, tung oil and many

others, it is very well suited to the production of Japanese sugar cane, as evidenced by plate 6, figure 2.

Portsmouth loamy fine sand retains moisture well during the dry seasons. Consequently it is well suited to the production of Japanese sugar cane which has a shallow root system and requires an enormous amount of water during the growing season. During the wet summer season the water table on this soil is within 12 to 18 inches of the surface, a condition which makes it entirely unsuited to the production of crops with a rangy root system.

Loamy phase of Leon fine sand soil rates as only a mediocre Irish potato soil, is a very poor soil for grapes, pecans, and several other crops but is very good as a sweet potato soil. The potatoes shown in plate 7, figure 1 all came from one hill of potatoes grown on loamy phase of Leon fine sand. This soil happens to possess the particular peculiarities necessary for a satisfactory sweet potato soil.

That peppermint is just as sensitive to a change in soil type as most of the other crops discussed, is clearly shown by plate 7, figure 2. The plants in the right foreground are on Cypress pond soil whereas those in the left background are on loamy phase of Leon fine sand. The last named soil is entirely too droughty in character to be a suitable soil for the production of peppermint whereas the Cypress pond soil contains the proper constituents to enable it to hold suitable moisture for a satisfactory growth of peppermint.

The data and photographs in this article make up only a small proportion of the total information collected on the point under discussion. However, these data are sufficiently conclusive to indicate the value of soil surveys in the economic development of Florida soils.

PLATE 1

GRAPE PLANTS SET MARCH 4, 1927, PHOTOGRAPHED JULY 21, 1927

FIG. 1. Grapes on Blanton fine sand.

FIG. 2. Grapes on Leon fine sand (loamy phase).



FIG. 1



FIG. 2

PLATE 2

PLANTS SET IN EARLY MARCH, 1927, PHOTOGRAPHED JULY 13, 1927

FIG. 1. Peaches, pears, and plums on Blanton fine sand.

FIG. 2. Pecans on Leon fine sand (loamy phase).



FIG. 1

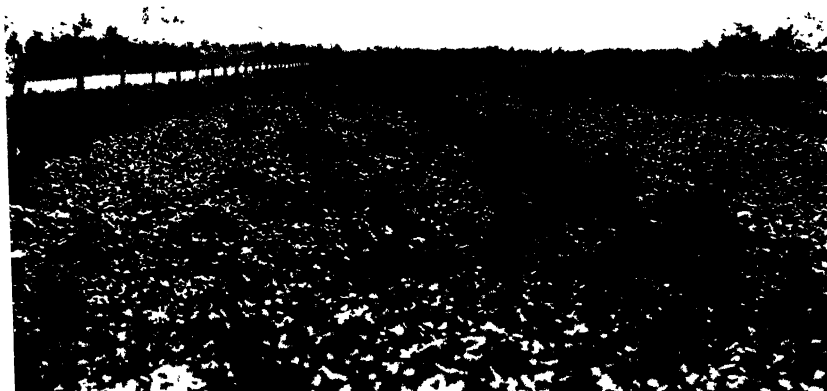


FIG. 2

PLATE 3

BLUEBERRIES; FOREGROUND LEON FINE SAND; BACKGROUND BLANTON FINE SAND

Plants all set at the same time and cultivated uniformly throughout



PLATE 4

FIG. 1. Tung Oil trees; left, Norfolk fine sand; right, Leon fine sand. Plants all set at the same time and cultivated uniformly throughout.

FIG. 2. *Crotalaria* on Leon fine sand. Photographed November 2, 1927.



FIG. 1



FIG. 2

PLATE 5

FIG. 1. Corn on Portsmouth Loamy fine sand. Photographed July 13, 1927.

FIG. 2. Corn on Leon fine sand. Photographed July 13, 1927.



FIG. 1



FIG. 2

PLATE 6

FIG. 1. Peppers on St. John's loamy fine sand. Plants set in April, 1927, photographed July 13, 1927.

FIG. 2. Japanese sugar cane on Portsmouth loamy fine sand. Photographed November 5, 1927, produced 40 tons to the acre.



FIG. 1

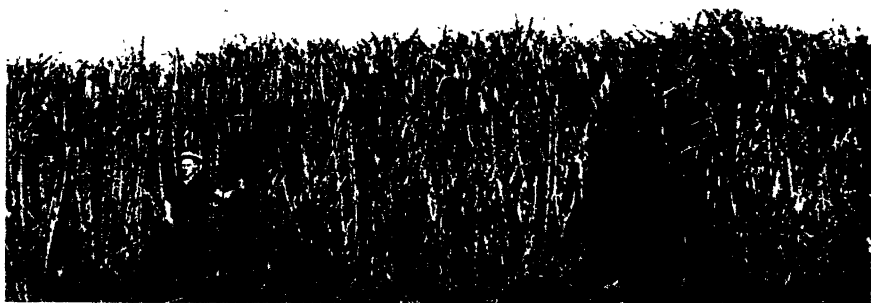


FIG. 2

PLATE 7

FIG. 1. Sweet potatoes grown on Leon fine sand, loamy phase. All from one hill.

FIG. 2. Peppermint; foreground, Cypress Pond soil; left background, Leon fine sand, loamy phase. Photographed July 13, 1927.



FIG. 1



FIG. 2

THE USE OF DYES IN THE ISOLATION OF A NITRITE OXIDIZING ORGANISM¹

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Although the process of nitrification in its many different aspects has been the subject of a great deal of study since the first observations of Schloessing and Müntz in 1878, the isolation of the nitrifying organisms still remains a difficult task in the hands of most laboratory workers. These bacteria are obligate autotrophic organisms possessing definite physiological characteristics which differentiate them from other bacteria. They develop in a pure mineral medium containing oxidizable inorganic substances which serve as the only source of energy. Organic nutrients are not necessary for growth or energy requirements. Such nutrients have been shown by numerous workers to exert a toxic effect on the nitrifying bacteria, especially when used in the amounts that are commonly employed in bacteriological media. By means of the energy secured from the oxidation of the inorganic nitrogenous compounds these organisms are able to reduce carbon dioxide, thus securing carbon from this source for the synthesis of their organic structures.

Advantage is taken of these physiological characteristics in isolating the nitrifying bacteria. By the use of an inorganic medium containing the desired oxidizable nitrogenous compound it is possible to eliminate from cultures of these organisms the majority of the soil flora, especially the obligate heterotrophic bacteria. It is an established fact, however, that certain organisms which are non-oxidizers of ammonium and nitrite compounds are capable of rapid growth in these highly specialized media.

HISTORICAL

Winogradsky (14) first reported the isolation of the nitrifying bacteria in 1890. Since that time many investigators have attempted to secure pure cultures of these organisms by following the technique developed by Winogradsky. A few of these workers have been successful in their efforts. Others have failed because of their inability to eliminate certain contaminating forms. In numerous instances investigators working with the nitrifying bacteria have proceeded with impure cultures because of the difficulties arising in attempting to isolate these organisms in pure cultures. Meek and Lipman (10), studying

¹ Published with the approval of the director of the Idaho Agricultural Experiment Station as scientific paper number 61

the relation of reaction and salt concentration of the medium on the nitrifying bacteria, used crude cultures. They make the following statement in regard to the use of such cultures: "Difficulties encountered in attempts to obtain pure cultures of the nitrifying bacteria led us to adopt the use of a crude, but invigorated culture in each case, which was produced by numerous successive transfers to fresh media from a strong soil culture in the solutions above described." Gowda (8) used cultures of the nitrifying organisms which had gone through a number of sub-cultures. He made no attempt to secure pure cultures of these organisms.

It is evident that further work is needed to develop a more desirable technique for the isolation of these bacteria than exists at the present time. This would permit studies to be made which would assist in the clarification of certain conceptions pertaining to the morphological and physiological characteristics of the nitrifying bacteria. The work reported in this paper is confined to a study of the isolation of a nitrite oxidizing organism and a discussion of the predominating contaminating forms.

The well-known fact that certain dyes exhibit a selective action toward various organisms suggested their use in this study as a means of freeing cultures of the nitrite oxidizing organism of bacteria that persist when other methods are used. The bacteriostatic action of dyes, especially the dyes of the tri-phenyl methane group, has received considerable attention. Churchman (4) demonstrated rather conclusively the bacteriostatic action of gentian violet toward cultures of *B. anthracis*. In a more recent study Churchman (5) has shown acid fuchsin to possess a bacteriostatic action the reverse of that possessed by gentian violet. Anderson (1), Batchelor and Curie (2), and Vandecaveye (12) have made use of dyes in the isolation of the root nodule bacteria of legumes.

METHODS

The first step in the isolation of the nitrite oxidizing organism is the securing of a crude culture of the organism which is comparatively free from other bacterial forms. This is accomplished by the enrichment process: i.e., the development of the organism in a medium suited to its particular requirements. Successive transfers to such a medium with the subsequent additions of nitrite after oxidation tend to increase the numbers of this organism accompanied by a decrease in the number of undesirable types. Many investigators have carried such cultures through numerous transfers with the hope of finally eliminating all contaminating forms, thereby securing a pure culture. This, however, has not proved successful. Gibbs (7) has shown these contaminating forms to persist after 35 successive transfers. Gowda (9) was unable to secure pure cultures by this method. He found certain contaminating forms to be present after many successive transfers.

The media used in the cultivation of the nitrite oxidizing organism were the same as those used by Gibbs. These media were prepared with conductivity

water and chemicals of the highest purity and were of the following composition:

Liquid medium

| | gm. |
|-----------------------------|--------|
| Sodium nitrite..... | 1.0 |
| Sodium carbonate..... | 1.0 |
| Di-potassium phosphate..... | 0.5 |
| Sodium chloride..... | 0.5 |
| Magnesium sulfate..... | 0.3 |
| Ferric sulfate..... | Trace |
| Water..... | 1000.0 |

Washed agar medium

1 cc. portions of each of the following solutions were added to 15 cc. of a 2 per cent washed agar:

| | gm. |
|---------------------------------|-------|
| (a) Di-potassium phosphate..... | 1.5 |
| Water..... | 100.0 |
| (b) Sodium nitrite..... | 1.5 |
| Sodium carbonate..... | 1.5 |
| Water..... | 100.0 |
| (c) Magnesium sulfate..... | 0.45 |
| Sodium chloride..... | 0.75 |
| Ferric sulfate..... | 0.02 |
| Water..... | 100.0 |

Twenty-five-cubic-centimeter portions of the liquid medium were dispensed into 200-cc. Erlenmeyer flasks and sterilized in the autoclave. The solutions used with the washed agar were prepared and sterilized separately. These were mixed with the washed agar at the time of pouring plates. One-cubic-centimeter portions of solution (b) were used as enrichment additions to cultures which had oxidized the nitrite.

A portion of soil, approximately 2 gm. in weight, was inoculated into a flask of the nitrite medium and incubated at 28°C. Oxidation of the nitrite usually was complete after a period of from 15 to 20 days. This was determined by the use of Tromsdorff's reagent. A negative test for nitrite indicated that the nitrite had been oxidized to nitrate. One loop of this actively oxidizing culture was transferred to a 25-cc. portion of sterile nitrite medium. When oxidation was complete another transfer was made. This process was continued until four successive transfers of the culture had been made. Nitrite was added to this culture at intervals of five to seven days, depending upon the rate of oxidation. At this stage the more common soil forms had been eliminated, but the culture was still impure as indicated by growth on plain agar plates.

Plain agar loop dilution plates made from this actively oxidizing culture showed the presence of numerous colonies of two types of organisms. One

organism was a gram-negative coccus, usually occurring in pairs and forming a small circular pink colony. The other organism was a small gram-negative, extremely motile bacillus. This organism formed a small circular yellow

TABLE 1
Effect of dyes on a crude culture of the nitrite oxidizing organism

| | DYE USED | MINUTES OF EXPOSURE | | | | | | | | |
|----------------|---------------------------------------|---------------------|---|---|---|---|----|----|----|----|
| | | 1 | 2 | 3 | 5 | 7 | 10 | 15 | 20 | 30 |
| Chemical group | Phenyl-methane: | | | | | | | | | |
| | Acid green 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Acid green 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Malachite green 0.5 per cent | + | + | + | + | + | - | - | - | - |
| | Malachite green 1.5 per cent | - | - | - | - | - | - | - | - | - |
| | Brilliant green 1.0 per cent | - | - | - | - | - | - | - | - | - |
| | Acid fuchsin 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Basic fuchsin 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Crystal violet 0.5 per cent | - | - | - | - | - | - | - | - | - |
| | Methyl green 0.125 per cent | - | - | - | - | - | - | - | - | - |
| | Methyl violet 0.5 per cent | - | - | - | - | - | - | - | - | - |
| | Rosaniline hydrochloride 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Xanthene: | | | | | | | | | |
| | Eosin 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Eosin B. 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Rose bengal 0.75 per cent | + | + | + | + | + | + | + | + | + |
| | Rose bengal 1.0 per cent | - | - | - | - | - | - | - | - | - |
| | Quinone-imide: | | | | | | | | | |
| | Methylene blue 1.0 per cent | - | - | - | - | - | - | - | - | - |
| | Methylene blue 0.5 per cent | + | + | - | + | + | - | + | - | - |
| | Thionine 1.0 per cent | + | + | + | - | - | - | - | - | - |
| | Neutral red 1.0 per cent | + | 0 | + | 0 | + | 0 | + | 0 | 0 |
| | Neutral red 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Azo: | | | | | | | | | |
| | Bismarck brown 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Orange G. 1.0 per cent | + | + | + | + | + | + | + | + | + |
| Control | | Oxidized | | | | | | | | |

+ Nitrite oxidized to nitrate.

- Nitrite not oxidized.

0 Not determined.

colony. A more detailed description of these organisms will be found later in this paper.

An experiment, preliminary in nature, was designed to test the effect of the dyes upon this impure culture. Two per cent aqueous solutions of dyes were prepared and designated as stock dyes. Solutions of the desired strength

were prepared from these stock dyes. The technique of the test consisted of adding four drops of the culture to 4 cc. of the dye solution in a test tube. The dye solution and culture were thoroughly mixed and one drop transferred to a flask of nitrite medium after intervals of 1, 2, 3, 5, 7, 10, 15, 20, and 30 minutes of exposure. To serve as a control four drops of the actively oxidizing culture were added to 4 cc. of sterile water. After mixing, one drop was transferred

TABLE 2
Growth in plain broth of cultures oxidizing nitrite

| | DYE USED | MINUTES OF EXPOSURE | | | | | | | | |
|----------------|---------------------------------------|---------------------|---|---|---|---|----|----|----|----|
| | | 1 | 2 | 3 | 5 | 7 | 10 | 15 | 20 | 30 |
| Chemical group | Phenyl-methane: | | | | | | | | | |
| | Acid green 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Acid green 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Malachite green 0.5 per cent | - | - | 0 | - | - | 0 | 0 | 0 | 0 |
| | Acid fuchsin 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Basic fuchsin 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Rosaniline hydrochloride 1.0 per cent | + | - | - | - | - | - | - | - | - |
| | Xanthene: | | | | | | | | | |
| | Eosin 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Eosin B. 1.0 per cent | + | + | + | + | + | + | + | + | + |
| | Rose bengal 0.75 per cent | + | + | + | + | + | + | + | + | + |
| | Quinone-imide: | | | | | | | | | |
| | Methylene blue 0.5 per cent | + | + | 0 | + | + | 0 | + | 0 | 0 |
| | Thionine 1.0 per cent | + | + | - | 0 | 0 | 0 | 0 | 0 | 0 |
| | Neutral red 1.0 per cent | + | 0 | + | 0 | + | 0 | + | 0 | 0 |
| | Neutral red 1.5 per cent | + | + | + | + | + | + | + | + | + |
| | Azo: | | | | | | | | | |
| | Orange G. 1.0 per cent | + | + | + | + | + | + | + | + | + |

to a flask of sterile nitrite medium. All flasks were incubated at 28°C. and tested for nitrite at intervals of four to five days during a 30-day incubation period.

RESULTS OF INVESTIGATION

Effect of dyes on oxidation

Table 1 shows the dye used, the time of exposure, and the relative bactericidal effect of the dye on the nitrite oxidizing organism, as determined by the inhibition of nitrate formation.

From table 1 it will be noted that a number of the dyes, in the concentrations used, inhibited the development of the nitrite oxidizing organism as indicated by the failure of the culture to oxidize nitrite.

Broth cultures

In the foregoing experiment all of the dye cultures which failed to show oxidation were discarded. Those which showed oxidation were transferred to plain broth in order to determine whether organisms were present which were capable of growing in that medium. According to Winogradsky the criterion of purity of the nitrifying bacteria is the ability to oxidize nitrite and the inability to develop in plain broth. The results of this experiment are shown in table 2.

The cultures which showed no growth when transferred to plain broth; namely, the cultures exposed to malachite green 0.5 per cent for 1, 2, 5, and 7 minutes; rosaniline hydrochloride 1.0 per cent for 2, 3, 5, 7, 10, 15, 20, and 30 minutes; and thionine 1.0 per cent for 3 minutes, fulfilled the criterion for purity as put forth by Winogradsky in his early studies of the nitrifying bacteria.

Plain agar plate cultures

It seemed desirable, however, to subject these cultures to a further test, especially one in which aerobic conditions would be at a maximum. For this purpose plain agar plates were used. These plates were streaked by the use of an inoculating machine as described by Varney (13). This method consists of applying the inoculum to the surface of the medium while the plate is rapidly rotating. This technique proved very desirable and many well-isolated colonies arranged in concentrics about the plate were secured. These plates were placed under a bell jar to prevent excessive evaporation during incubation. After a period of 15 days many very small white colonies, which were almost invisible to the naked eye, appeared on the surface of the medium. These colonies were examined under the low power objective of the microscope and were found to be all of the same type. On plain agar plates they varied from very small to 0.15 mm. in diameter. The centers of the colonies appeared quite dark with a gradual fading to the edge, which was irregular and lacerated. The organisms of these colonies stained readily with the ordinary bacteriological dyes, being stained especially well with cold carbol fuchsin when applied for only a few seconds. When stained by Gram's method they retained this stain. Carbol fuchsin stained preparations showed these organisms to possess a flagellum-like attachment at one pole, which in many instances was several times as long as the body of the cell. Colonies of this organism when transferred to a nitrite medium failed to produce oxidation of the nitrite.

Washed agar plate cultures

Washed agar plates were inoculated from the same cultures in a similar manner and incubated under a bell jar at a temperature of 28°C. After about 15 days many very small white colonies were visible to the naked eye. When

examined under the low power objective of the microscope two distinct types of colonies were observed, one type being morphologically identical with the colonies which developed on the plain agar plates, while the other type was at first rather irregular in shape, but as the colony grew larger and older it became round or oval and possessed a smooth entire edge and a finely granular internal structure. Under the microscope, colonies of the latter type appeared as light brown colonies and varied from extremely small to 0.2 mm. in diameter. On all washed agar plate cultures containing colonies of this type oxidation of the nitrite took place.

Nitrite oxidizing organism

Cold carbol fuchsin applied for only a few seconds proved to be the most desirable stain for demonstrating the morphological characteristics of the nitrite oxidizing organism. In stained preparations it occurs singly, in pairs, and in irregular clusters. It stains evenly, varies from oval to spherical in shape, the majority of the cells being spherical, and is from 0.8 to 1.0 μ in diameter. It is non-motile and retains Gram's stain when stained by that method. The morphology of the nitrite oxidizing organism is such that it is rather difficult to determine whether it should be classed as a coccus or as a rod form.

Separation of organisms on washed agar plate cultures

The two types of colonies appearing on washed agar plates were so small and so numerous that it was necessary to resort to the use of a modified Barber pipette in order to obtain pure cultures of these organisms. This was carried out in the following manner. Portions of agar were removed from the petri dish to a sterile cover slip, which was then inverted and supported under the low power objective of the microscope in such a manner that individual colonies could be examined. Colonies were selected and fished from the surface of the medium by the aid of a mechanically operated sterile glass pipette inserted from underneath the inverted colonies and slowly raised until contact with the desired colony was made. By this means pure cultures of each type of organism were secured and grown both in liquid and on solid media.

Oxidation of ammonia and nitrite

Throughout the course of this study a great many colonies of both types were transferred to nitrite agar and to a nitrite liquid medium. In almost all cases cultures originating from colonies possessing the smooth entire edge were capable of nitrite oxidation, while the cultures developing from the other type of colony, although growth from these transfers was quite evident as determined by microscopic and macroscopic examination, were not capable of producing nitrate from nitrite. It was thought desirable to test the ability of these two organisms to oxidize ammonium compounds to nitrite. Accordingly

numerous colonies of each organism were transferred to flasks of a sterile medium containing oxidizable nitrogen in the form of ammonium sulfate. After from 20 to 30 days microscopic examinations and chemical tests for the presence of ammonia and nitrite were made. In no case did cultures of either organism oxidize the ammonium compound to compounds of nitrite. Microscopic examinations of the cultures originating from the irregular type of colonies revealed the presence of large numbers of organisms in the ammonium medium. The fact that this organism grew very abundantly in both the ammonium and nitrite media without the oxidation of these compounds is sufficient evidence to eliminate it as belonging to the nitrifying group of bacteria.

Non-nitrite oxidizing organism

In numerous instances the polar flagellum-like attachment, which was noted when this organism was stained with cold carbol fuchsin, appeared to join a smaller deeply stained body to the bacterial cell. The length of this attachment varied on different cells. On many cells it was from 7 to 10 μ long, whereas on others it was much shorter. In some preparations the organisms were clustered together with the flagella-like attachments radiating away from the center of the cluster of bacteria. In many instances the cells were unevenly stained, there being an area at one end of the cell, sometimes near the flagellum-like attachment and sometimes at the other end, which would not take the stain. These cells were generally oval and somewhat pointed at the ends. They varied from 0.5 to 1.0 μ in width and from 1.2 to 1.8 μ in length. Among the early investigators who observed in their cultures an organism of similar description were Stutzer and Hartleb (11). They gave the name *Hypomicrobium* to this non-nitrifying organism. The greatest morphological difference these investigators found between *Nitrobacter* and *Hypomicrobium* was the thread-like growth from one pole of the *Hypomicrobium*.

Gibbs noticed a very short stem-like growth in many stained preparations of his pure cultures of *Nitrobacter*. Such growth was very noticeable when the preparation was treated by any method of staining flagella, but was seldom seen in the ordinary stained preparations. Gibbs found surface colonies on washed agar plates to occasionally appear regular in outline but more often irregular and spreading. This organism was distinctly oval in shape, commonly found in single cells or in pairs, about 0.6 to 0.8 μ wide and 1.0 to 1.2 μ long. Washed agar surface colonies of the nitrite oxidizing organism isolated in this study differ to some extent from washed agar colonies of the organism described by Gibbs. In almost all cases they appeared after 15 to 20 days as regular round or oval colonies possessing an entire edge and a finely granular internal structure. This was especially true in the case of well-isolated colonies but on thickly seeded plates there was a greater tendency toward irregular colonies.

Fred and Davenport (6) isolated and described an organism which appears

to be morphologically identical with the non-nitrifying organism isolated and described in this study. They found this organism to be capable of oxidizing nitrite to nitrate. They refer to it as *Nitrobacter*. In stained preparations they found this organism to possess a flagellum-like attachment which could be demonstrated when the organism was stained with cold carbol fuchsin or with Loeffler's flagella stain. One of the most characteristic arrangements in stained preparations was that of clumps of zooglea-like masses with only a few loose cells in the field. Some mounts showed the cells scattered more or less evenly over the field. They found the cells to stain unevenly, the center or more often one end of the cell being well stained, while the remainder, except the outline of the cell wall, did not take the stain at all. The microscopic appearance of stained preparations of the non-nitrifying organisms encountered in this study was characteristic in all details with the photomicrographs prepared and published by these investigators. The non-nitrifying organism of this study was found rather often in such a characteristic grouping as illustrated by a photomicrograph and a drawing in the paper of Fred and Davenport.

Fred and Davenport gave no detailed colony characteristics of the organism they have designated as *Nitrobacter*. From the rather striking morphological differences of the organism designated by Gibbs as being *Nitrobacter* and the organism described by Fred and Davenport, especially in the light of this study, it seems quite probable that these investigators were dealing with two different organisms.

Terminology used

In this study it has not seemed desirable to use the rather common term, *Nitrobacter*, in reference to the nitrite oxidizing organism. Neither has it appeared desirable, at this time, to classify this organism according to the terminology used in Bergey's *Manual of Determinative Bacteriology* (3) for those organisms capable of nitrite oxidation. Sufficient evidence has not been secured in this study definitely to classify this organism as being *Nitrobacter winogradskyi* Buchanan, or as being *Nitrosococcus nitrosus* (Migula) Bergey et al. For these reasons reference has been made to this organism throughout this paper as "the nitrite oxidizing organism."

Further experiments

Experiments testing the effect of malachite green, thionine, and rosaniline hydrochloride on crude cultures of the nitrite oxidizing organism were repeated many times. The results with malachite green and thionine were not generally favorable, as these dyes did not always check the growth of the contaminating forms. In numerous cases these forms were found to resist greater concentrations of the dyes than did the nitrite oxidizing organism. Very satisfactory results were usually obtained with the use of rosaniline hydrochloride, a tri-phenyl methane derivative. This dye proved to be a very satisfactory agent in eliminating the two most objectionable contaminating

forms, especially when used in 1.0 per cent concentrations and with exposures varying from 5 to 30 minutes.

Contaminating forms

In this study three contaminating bacterial forms were encountered, one of which has been described in detail. The other two organisms were found to grow very rapidly in the inorganic medium which was employed in this study as well as in ordinary nutrient media. The description of these organisms is as follows:

COCCUS FORM

Morphology. In 24-hour cultures on agar slants this organism appears as a coccus and is usually found in pairs. It varies from 0.8 to 1.0 μ in diameter. It is non-motile, non-spore forming, and reacts negatively when stained by Gram's method. It stains readily with the ordinary dyes and can best be studied when stained with carbol fuchsin for about 30 seconds.

Cultural characteristics. Growth on plain agar slants is smooth, raised, and characterized by a glistening surface. Agar plate surface colonies are circular, raised, finely granular, and possess an entire edge. Surface colonies may develop to as large as 2 or 3 mm. in diameter. Colonies within the medium assume a lens-shaped appearance. Both surface and deep colonies on forming are orange, and become pink as the colony ages.

Physiology. This organism grows well on the surface of nutrient agar, on gelatin, and in nutrient broth. It does not liquefy gelatin, reduce nitrate, nor possess diastatic properties. Litmus milk becomes alkaline in reaction after the second day, although the alkalinity is not marked. Litmus is not reduced. Dextrose is fermented with the production of acid but no gas. Lactose, sucrose, and glycerin are not attacked. In lactose, sucrose, and glycerin broths the alkalinity increases until at the end of one week's incubation a pH value of 8.0 is reached. Indol is not produced.

ROD FORM

Morphology. Stained preparations from 24-hour agar cultures of this organism appear as small rods, occurring singly, and varying from 0.3 to 0.5 μ in diameter and from 0.8 to 1.2 μ in length. It is extremely motile, non-spore forming, and reacts negatively when stained by Gram's method. It stains readily with the ordinary bacteriological dyes.

Cultural characteristics. Moderate growth occurs on plain agar slants and in plain broth. Growth on agar slants is smooth, slightly raised, and glistening. Surface colonies are circular, finely granular, entire edge, raised, and may reach a diameter of 3 to 4 mm. Deep colonies are lens-shaped. This organism produces a yellow pigment in plain agar.

Physiology. Growth takes place readily on the surface of nutrient agar, on gelatin, and in nutrient broth. It does not form indol, reduce nitrate, liquefy gelatin, nor exert a diastatic action. Alkalinity in litmus milk is very pronounced. The entire tube of litmus milk except the surface is reduced at the

end of a 7-day incubation period. It does not attack dextrose, lactose, sucrose, nor glycerin. After 24-hours' incubation these sugar broths develop an alkaline reaction which reaches a maximum pH value of 8.2 to 8.4 after seven days of incubation.

Actinomyces were encountered in most of the washed agar plate cultures made from liquid cultures which had been exposed to the action of dyes. In a few instances these organisms were troublesome contaminants. In most cases, however, detailed colony study of the nitrite oxidizing organism and of the contaminating bacterial forms as well as isolation work could be carried on without a great deal of interference from these organisms.

SUMMARY

Numerous dyes of the phenyl-methane, xanthene, azo, and quinone-imide groups have received attention in this study. The possibilities of all of these dyes have not been exhausted, but most of them were discarded in order to permit concentration on a few of the most promising. Of this selected group of dyes rosaniline hydrochloride, a tri-phenyl methane derivative, has given the most favorable results. The two most objectionable contaminating forms can be eliminated by exposing crude cultures of the nitrite oxidizing organism to the action of this dye in 1.0 per cent concentrations for from 5 to 30 minutes. The other contaminating form can be eliminated by the use of mechanically operated sterile minute glass pipettes in fishing colonies from the surface of a solid medium.

A detailed comparison was made of the nitrite oxidizing organism and of the contaminating form which could not be eliminated by the use of rosaniline hydrochloride. This contaminating form is capable of rapid growth in nitrite medium, but only a very scanty growth on a nutrient agar. This organism is not capable of oxidizing ammonia to nitrite or of oxidizing nitrite to nitrate. It is of interest in this study because certain investigators have described an organism having similar morphological characteristics as being *Nitrobacter*. This organism stains well with cold carbol fuchsin and when stained by this dye many of the cells appeared to possess a flagellum-like attachment at one pole which in many instances was several times as long as the body of the cell. In some preparations the organisms were clustered together with the flagellum-like attachments radiating away from the center of the cluster of bacteria. In many instances the cells were unevenly stained and somewhat pointed at the ends. This organism forms a colony which can be differentiated from the colony formed by the nitrite oxidizing organism by its characteristic appearance and unevenness of edge.

In stained preparations the nitrite oxidizing organism appears singly, in pairs, and in irregular clusters. It stains evenly and varies from oval to spherical in shape. The morphology of the nitrite oxidizing organism is such that it is difficult to determine whether it should be classed as a coccus or as a rod form.

In this study it seemed desirable to refer to this organism as "the nitrite oxidizing organism" rather than to use the term *Nitrobacter* or the terminology suggested in Bergey's *Manual of Determinative Bacteriology* for those organisms oxidizing nitrite to nitrate. In the opinion of the writer the term *Nitrobacter* has been used by various workers in reference to entirely different organisms.

A detailed study was made of the morphological, physiological, and cultural characteristics of the two contaminating forms which were capable of rapid growth on ordinary culture media. These organisms are pigment-formers. One organism is a gram-negative coccus, usually occurring in pairs and forming a small circular pink colony. The other organism is a small Gram-negative, extremely motile bacillus, forming a small circular yellow colony. Both organisms were capable of rapid growth in an inorganic nitrite medium but neither was capable of oxidizing the nitrite to nitrate. By eliminating these forms from cultures of the nitrite oxidizing organisms the process of isolation is greatly simplified.

Although actinomycetes were encountered in this study these organisms did not interfere to any great extent with the isolation of the nitrite oxidizing organism or with the study of the contaminating bacterial forms.

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THE VERTICAL DISTRIBUTION OF SOIL BASES AND ACIDITY IN SOME ILLINOIS SOILS¹

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Soil profile studies are fruitful fields of investigation, and offer much promise for the future. If our understanding of soils is to be enhanced, it must come largely through greater accuracy in observations and refinement in measurements of the physical, chemical, and biological properties of the constituents of the soil profile. Much effort has been devoted to such refinements in technic in recent years.

The variations found in the profiles of soils are due principally to differences in age, rainfall, temperature, topography, and parent material. To determine how some Illinois soil profiles differ in certain chemical aspects with particular reference to reaction, base exchange, and distribution of calcium and magnesium is one of the objects of this investigation: a second objective is to study the influence of soil treatment upon these soils; and a third, to study the relation between the chemical constitution and the crop yields.

No complete review of literature is attempted in this paper, because many such reviews are already available. Among these may be mentioned the following: Way (32); Johnson (19); Gedroiz (14); Ames and Schollenberger (1); Noyes (24); Prescott (28); Fisher (12); Crowther (8); Gehring (15); Askinasi (2).

With respect to chemical studies on Illinois soils, Catherwood and DeTurk (6) made some investigations on the relation of soil treatment to exchangeable calcium and magnesium in four southern Illinois soils. They also compared soil types in regard to exchangeable and total bases, using four soils of greatly differing stages of maturity. Their findings will be referred to in connection with the results obtained in this study.

The plan of attack was to sample several soil types by 3- to 4-inch layers, using care not to include material from more than one horizon in any layer-sample. Samples were taken from fertilized as well as untreated areas. These were studied by means of the following determinations: Comber test, lime-

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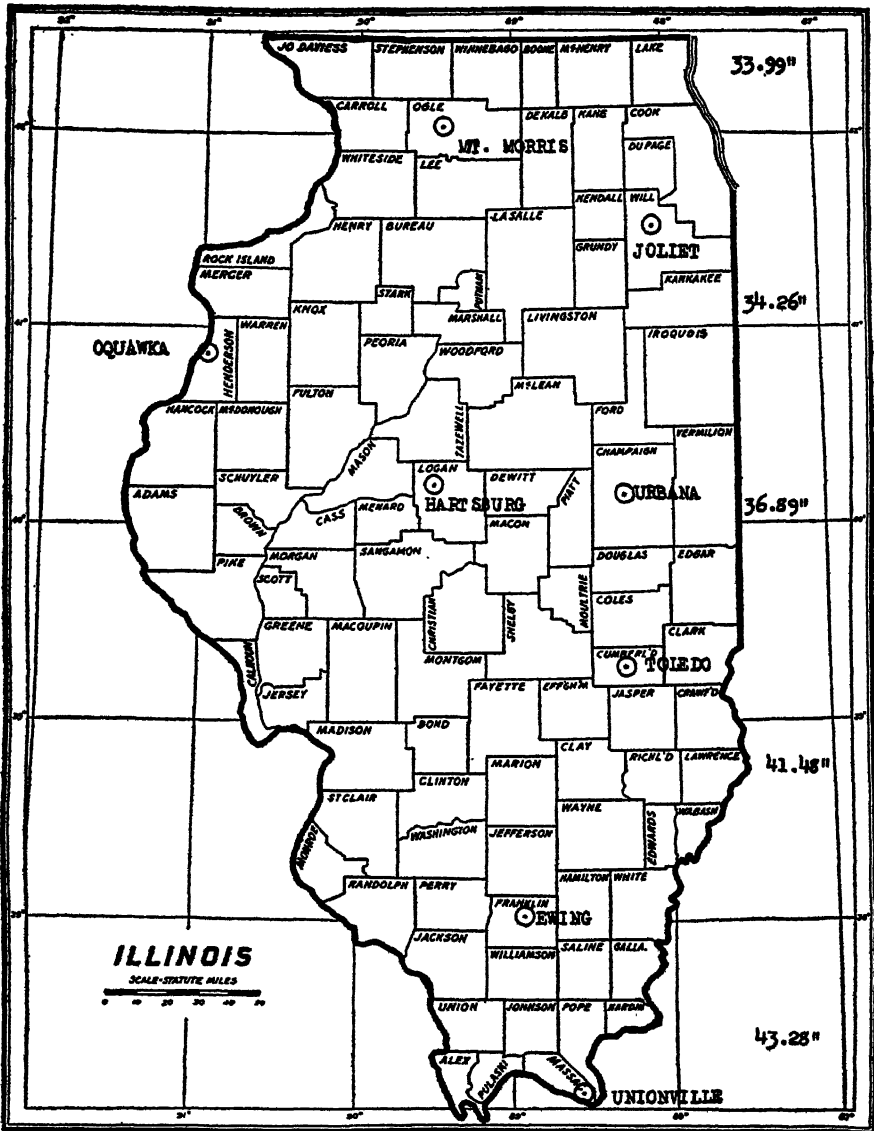


FIG. 1. MAP SHOWING LOCATION OF EXPERIMENT FIELDS FROM WHICH SAMPLES WERE TAKEN

On the right is indicated the average annual rainfall of the several latitudinal divisions of the state.

requirement by the Hutchinson-MacLennan calcium bicarbonate method and the Hopkins potassium nitrate method, hydrogen-ion concentration, buffer action, total calcium and magnesium, exchangeable calcium and magnesium, and the correlation of some of these results with crop yields.

DESCRIPTION OF SOILS, SOIL TREATMENTS, AND METHODS OF SAMPLING

The soils used in this investigation were taken from eight of the Illinois experiment fields, the basis of selection being (a) soil type, and (b) response to liming as indicated by crop yields. Samples were taken by horizons. In the case of horizons A₂ and B, if either was six inches or more thick, it was divided into two or more strata of three to four inches each. The C horizon was sampled only where it occurred within 25 to 30 inches of the surface.

Each sample was a composite of 18 borings made with the ordinary screw-type auger. Some objection may be made to the use of the auger for sampling these comparatively thin strata, and the author recognizes the limitations of this method. It would be more desirable to use a spade, making an excavation large enough to permit the observer to examine the undisturbed soil and to sample the accurately measured strata. This, of course, could not be done on the experimental plats where the study of the effect of treatment is to be continued. Moreover, a single sample taken by excavation would not necessarily represent the plat as a whole any more accurately than a composite made up of an adequate number of borings.

The location of these fields in the state is shown in figure 1. As indicated on the map, there is a gradual increase in the total annual rainfall southward (22).

All but one of the soils used in this study occur within the glaciated region. The extreme southern portion of the state in which the Unionville field is located is one of the few, small, unglaciated areas to be found in Illinois.

The profile descriptions of the soils studied and the sampling depths are as follows:

1. Ewing field (established 1910)

Gray Silt Loam On Orange-Mottled Tight Clay

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION* |
|------------------------------------|--|
| A ₁ —0 to 8" | Brownish gray silt loam |
| A ₂ —8 to 12" | Slightly mottled, brownish gray silt loam containing some yellow spots |
| A ₃ —12 to 15" | Gray silt loam heavily spotted with orange red |
| B _{1a} —15 to 18" | } Highly plastic, gray clay heavily spotted with orange red |
| B _{1b} —18 to 21-22" | |
| C ₁ —21 to 24-25" | Friable, gray silty clay loam heavily spotted with yellowish brown. |

Crop response to liming—R:RL = 100:352†.

* The profile description in each case has been taken from Illinois Bulletin 273 (3).

† Based on total dry weight yields for 10-year period, 1917-1926. R = residues; RL = residues + limestone.

2. Toledo field (established 1913)

Gray Silt Loam On Tight Clay

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|------------------------------------|---|
| A ₁ —0 to 8" | Brownish gray silt loam Gray silt loam |
| A _{2a} —8 to 12" | |
| A _{2b} —12 to 16" | |
| B _{1a} —16 to 19-20" | Strongly mottled, yellowish brown, highly plastic clay |
| B _{1b} —19-20 to 23-28" | |
| C ₁ —23-28 to 27-33" | |
| | Gray or drabish gray, friable silty clay loam containing many yellowish brown and black iron splotches. |

This type is characterized by a great variation in the depth of the B, or compact, horizon. Crop response to liming,—R:RL = 100:223.

3. Unionville field (established 1911)

Yellow-Gray Silt Loam

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|------------------------------------|--|
| 1927 samples | |
| A ₁ —0 to 7" | Grayish yellow silt loam Yellowish gray silt loam |
| A _{2a} —7 to 12" | |
| A _{2b} —12 to 15" | |
| A _{2c} —15 to 19" | Compact, mottled, bright yellow silty clay loam. |
| B _{1a} —19 to 24" | |
| B _{1b} —24 to 28" | |

1925 samples: Depths A₁—0 to 7"; A₂—7 to 20".

Crop response to liming,—R:RL = 100:151.

4. Oquawka field (established 1915)

Dune Sand, Terrace (Plainfield sand)

"The surface is light brown between dunes, and yellowish brown on the tops of the dunes. The depth of the surface varies; it frequently is 15 inches deep between the dunes and may be entirely absent on top of the dunes. There is no horizon development below the surface, or A₁ horizon, the material consisting of incoherent yellow sand."

Sampling depths:

- | | |
|-------------------------------|----------------|
| 1. (A ₁)— 0 to 8" | 4.—16.5 to 21" |
| 2. — 8 to 12" | 5.—21 to 24" |
| 3. —12 to 16.5" | 6.—24 to 27" |

Crop response to liming,—R:RL = 100:306.

5. Joliet field (established 1914)

Brown Silt Loam On Calcareous Drift (Clarion silt loam)

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|------------------------------------|--|
| A ₁ —0 to 8" | Brown silt loam |
| A _{2a} —8 to 12" | Light brown or yellowish brown silt loam |
| A _{2b} —12 to 15" | |
| A _{2c} —15 to 19.5" | |
| B ₁ —19.5 to 24" | Yellowish brown, medium-compact clay, or sandy and gravelly clay |
| C ₁ —24 to 27" | Yellow, highly calcareous, sandy, and gravelly drift. |

(On the Joliet field the surface soil is lighter colored, and the C horizon is reached at a shallower depth than is usual for the type).

Crop response to liming,—R:RL = 100:105.

6. Mt. Morris field (established 1910)

Light Brown Silt Loam (Tama silt loam)

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|------------------------------------|---|
| A ₁ —0 to 8" | Light or yellowish brown silt loam |
| A _{2a} —8 to 12" | Distinctly yellowish brown, friable silt loam |
| A _{2b} —12 to 16.5" | |
| B _{1a} —16.5 to 21" | Brownish yellow, friable, non-mottled, non-compact silt loam |
| B _{1b} —21 to 24" | |
| C ₁ —24 to 27" | Very friable, slightly mottled, bright yellowish brown silt loam. |

Crop response to liming,—R:RL = 100:143.

7. Urbana field (Davenport plots, established 1895)

Brown Silt Loam (Muscatine silt loam)

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|------------------------------------|--|
| A ₁ —0 to 8" | Brown silt loam |
| A _{2a} —8 to 12" | Light brown or yellowish brown silt loam |
| A _{2b} —12 to 15" | |
| A _{2c} —15 to 18" | |
| B _{1a} —18 to 22.5" | Mottled, pale yellow, medium-compact clay loam or silty clay loam. |
| B _{1b} —21.5 to 26–27" | |

crop response to liming

| | |
|------------------------|------------------|
| Series 100E | R:RL = 100:125 |
| Series 200 W | R:RL = 100:109 |
| Series 200 E | (Not calculated) |
| Series 300 W | R:RL = 100:141 |
| Series 400 E | R:RL = 100:115 |

8. Hartsburg field (established 1911)
Black Clay Loam (Grundy clay loam)

| HORIZONS AS SAMPLED FOR THIS STUDY | DESCRIPTION |
|---------------------------------------|---|
| A ₁ —0 to 8" | Black clay loam |
| A _{2a} —8 to 12" | } Grayish or drabbish brown clay loam |
| A _{2b} —12 to 16" | |
| A _{2c} —16 to 21" | |
| B _{1a} —21 to 25" | } Gray clay loam splotted with black iron concretions and yellow mottling, not very compact or plastic. |
| B _{1b} —25 to 29" | |

Crop response to liming,—R:RL = 100:102.5.

The Urbana, Mt. Morris, Joliet, Ewing, and Oquawka fields were sampled between August 15 and September 15, 1926; Hartsburg and Toledo, May 1927; Unionville, April, 1925, and August, 1927.

Soil treatments

For those portions of the experimental fields used in this study the standard plan of soil treatments is as follows:

Plot 6 R residues
 7 RL residues and limestone
 8 RLP residues, limestone and phosphate
 9 RLPK residues, limestone, phosphate and potash
 10 0 check

This is true for each field except Urbana, where the arrangement is:

Plot 1 0 check
 2 R residues
 4 RL residues and limestone
 6 RLP residues, limestone, } bone phosphate E half
 } rock phosphate W half
 8 RLPK residues, limestone, potash } bone phosphate E half
 } rock phosphate W half

The total amount of limestone that has been applied to the soils varies from 13,000 to 20,000 pounds per acre.

METHODS OF ANALYSIS

Comber potassium thiocyanate test

Approximately 2 gm. of 10-mesh soil (measured) and 5 cc. of alcoholic potassium thiocyanate (40 gm. thiocyanate in a liter of 95 per cent alcohol) were vigorously shaken together in a test tube for a few seconds, and the readings taken after about 15 minutes. It was found that it is very essential to avoid grinding the samples for this test.

Hutchinson-MacLennan lime requirement method

Ten grams of air-dry, 10-mesh soil were shaken one hour with 100 cc. of approximately 0.017 *N* calcium bicarbonate, precautions being taken to keep an abundance of carbon dioxide present in order to prevent possible precipitation of calcium carbonate. The suspension was filtered and a 50-cc. aliquot titrated against 0.1 *N* acid.

Hopkins lime-requirement test

A 100-gm. sample of 10-mesh soil was shaken for one hour with 250 cc. of normal potassium nitrate solution. After settling, a 125-cc. portion of the supernatant liquid was drawn off and titrated against 0.1 *N* sodium hydroxide. The titration figure was multiplied by the factor 2.5 (18), and the result presented as grams of calcium carbonate required by 100 gm. of soil.

Hydrogen-ion concentration

The hydrogen-ion concentration, expressed as pH, was determined electrometrically, the hydrogen electrode (4) being used. One hundred mesh soil was used throughout, with the exception of the Oquawka sand, which was used in the unground condition. All determinations were run in duplicate.

Indications from observations of the author and others are that the grinding of a soil increases the pH value slightly in the silt loams and clay loams, and considerably in the case of sands.

Buffer action

Ten-gram samples of 20-mesh soil were shaken one hour with varying amounts of 0.038 *N* calcium hydroxide and enough water to make 20 cc. of liquid. At the end of that period the pH was determined, using the quinhydrone electrode. This method gave very satisfactory results with all except the Unionville soil. In the latter case, the results were nearly 2 pH higher than the figure obtained by the hydrogen electrode. Repeated trials with other samples from the same field gave the same results. This brings out the weak point in the quinhydrone method—occasionally it will give erratic results. Final data on this soil were secured by using the hydrogen electrode. Buffer action was determined only in the A₁ horizon of the unlimed soils.

Total calcium and magnesium

The estimation of calcium and magnesium was made in accordance with standard methods, using the following general procedure: Fusion of the 100-mesh soil with Na₂O₂; double precipitation of R₂ O₃; single precipitation of CaC₂O₄, which was titrated with KMnO₄; and double precipitation of the MgNH₄PO₄ which was ignited to Mg₂P₂O₇. All samples were analyzed in duplicate.

Exchangeable calcium and magnesium

Because of the large number of samples to be analyzed in the limited time available, it was necessary to choose a method that would not be too cumbersome and time-consuming. Furthermore, as some of the samples contained carbonates, the solubility of these carbonates in the displacing solution had to be taken into consideration. Normal solutions of potassium chloride and ammonium acetate, and a 0.05 *N* solution of hydrochloric acid were tried as displacing agents, with more or less success. Although the solutions varied in their ability to dissolve carbonates, all of them took into solution large enough amounts—especially from the Joliet subsoil which was particularly calcareous—to give high results for calcium. In order to correct for the amount of calcium carbonate dissolved, it was necessary to determine the carbonates in the soil before and after leaching.

Procedure adopted for determining exchangeable calcium and magnesium. Twenty-five grams of air-dried soil were placed in a beaker, and about 150 cc. of normal ammonium acetate added. This was stirred several times, and after four or five minutes the mixture was transferred quantitatively to a 10-cm. Buchner funnel, using a good grade of ordinary rapid filter paper such as S & S 589 white ribbon, and using light suction. A total of 750 cc. was passed through the soil in small portions, the filtrate evaporated to dryness, the acetate driven off and the organic matter partially removed by ignition on a silica plate over a Bunsen burner. The remaining organic matter was further treated with aqua regia, followed by dehydration.

The R_2O_3 compounds were removed with ammonium persulfate and ammonium hydroxide, and the calcium and magnesium determined, using double precipitation in each case. All extractions were made in duplicate, with single determinations on each extract.

In the first few sets, the R_2O_3 precipitate was ignited, weighed, and recorded. The amounts obtained, however, were consistently low.

Where carbonates were present in measurable quantities they were determined by the use of HCl in the Parr apparatus, before and after leaching the soil with ammonium acetate. The carbonate calcium dissolved by the salt solution was subtracted from the total amount of calcium in the filtrate.

This method of correcting for carbonates dissolved is not without fault, because of the depressing influence of calcium in solution upon calcium replacement. Where both calcium carbonate and magnesium carbonate are present the method cannot be used with any degree of accuracy, since there is no satisfactory way to distinguish between the quantity of the two kinds of carbonate in the soil, or between the amount of each dissolved and the amount of calcium and magnesium that is being replaced. MacIntire (21) presented data to show the non-existence of magnesium carbonate in humid soils.

In this investigation it is assumed that, except in the subsoil of the Joliet fields, no magnesium carbonate was present in these soils.

In the case of the Joliet subsoil samples the amount of carbonate found by analysis exceeded (when calculated as calcium) the amount of total calcium in the soil. Therefore, we are led to believe that magnesium carbonate does exist as such in this soil. It should be noted also that the total magnesium content of this stratum is very high. The data for exchangeable calcium in

the Joliet subsoil were obtained by assuming the amount of exchangeable calcium of the C₁ horizon to be commensurate with that found in the overlying B₁, which contained only a small amount of carbonate.

The experience of the writer in respect to the carbonate question bears out the accuracy of Gedroiz's observation: "Quite a considerable number of soils that we have investigated containing either CaCO₃ alone, or CaCO₃ and MgCO₃, lead us to the conclusion that, without further development of the methods for these cases, it is very risky to base any conclusions on the data for zeolitic Ca and Mg obtained by the method described here"³ (13).

In analyzing the soils for inorganic carbonates with the Parr apparatus, using 1:1 hydrochloric acid as the decomposing agent, it was observed that carbon dioxide was obtained from some of the most acid samples. This occurred particularly in the upper horizons of the Unionville and Mt. Morris soils. Both phosphoric and sulfuric acids were tried with identical results. Acetic acid, however, liberated no carbon dioxide from the acid soils, and in the case of limed soils gave slightly lower results than did hydrochloric acid.

It was found that acetic acid was just as effective as hydrochloric acid in decomposing calcium carbonate. Apparently hydrochloric acid reacts on the soil in a way different from acetic acid. Whether or not it is an organic decomposition is not known. Hissink (17) was confronted by the same problem, having obtained carbon dioxide from a soil with a pH value of 4.58. Since his soils were very rich in organic matter he suggests that possibly a small amount of carbon dioxide is formed by oxidation of the carbon. However, in the case of the Illinois soils this liberation of carbon dioxide is not correlated with the amount of organic matter in the soil. Neither is it the result of boiling the suspension containing hydrochloric acid, since the same amount of carbon dioxide was obtained in the cold under reduced pressure.

PRESENTATION OF DATA

The data obtained in this investigation will be presented under the following headings: Comparison of Soil Types; Comparison of Soil Treatments; Correlation of Crop Yields with Laboratory Results.

Comparison of soil types

The untreated plots only are considered in the comparison of soil types. The results are shown in figures 3, 4, and 5. For the most part the data have been taken from plot 10 on each field excepting Urbana, where no. 1 is the untreated plot. In a few instances not all of the data were available for plot 10, and in these cases the information was taken from the residue plot, no. 6, on which, as on plot 10, no minerals have been applied.

³ Gedroiz refers to the determination of carbonates before and after leaching, and subtracting the calcium equivalent of the amount dissolved from the amount of calcium found in the filtrate.

Hydrogen-ion concentration.—The hydrogen-ion concentration decreases with the increasing depth in every case, although the decrease is very slight in the Mt. Morris and the Oquawka soils. These two soils exhibit considerable uniformity throughout their profiles in comparison with the other soils studied in this work. The subsoil on the Joliet field is markedly calcareous, which fact accounts for the great decrease in hydrogen-ion concentration.

It is interesting to note that the H-ion concentration of the lower strata of the Toledo soil,—Gray Silt Loam on Tight Clay,—is considerably less than that of the corresponding layers in the Ewing soil,—Gray Silt Loam on Orange-Mottled Tight Clay.

The low H-ion values in the lower levels of both of these soils indicate a "slick spot" conditions, since all soils examined in this region except "slick spots" have definitely higher H-ion concentrations in the B and C horizons.⁴ The lower H-ion concentration in the Toledo soil indicates a more pronounced development of "slick spot" formation. Although the amounts of replaceable calcium and "magnesium" are apparently sufficient to account for the reaction as found, these "slick spots" are characterized by the presence of replaceable sodium as well. Qualitative tests on these samples showed fairly large amounts of sodium.

Comber test.—In a very general way, the intensity of color of the Comber test follows the pH values. This shows that the Comber test is a remarkably good indicator of the H-ion concentration of the soil, and it can usually be relied upon qualitatively.

Buffer action.—Although the H-ion concentration is the most reliable measure of the acidity of the soil, it does not indicate the amount of lime that is necessary to bring an acid soil to the neutral point. This latter value may be determined with considerable accuracy by an electrometric titration of the soil with a base, as has been described under "Methods of Analysis."

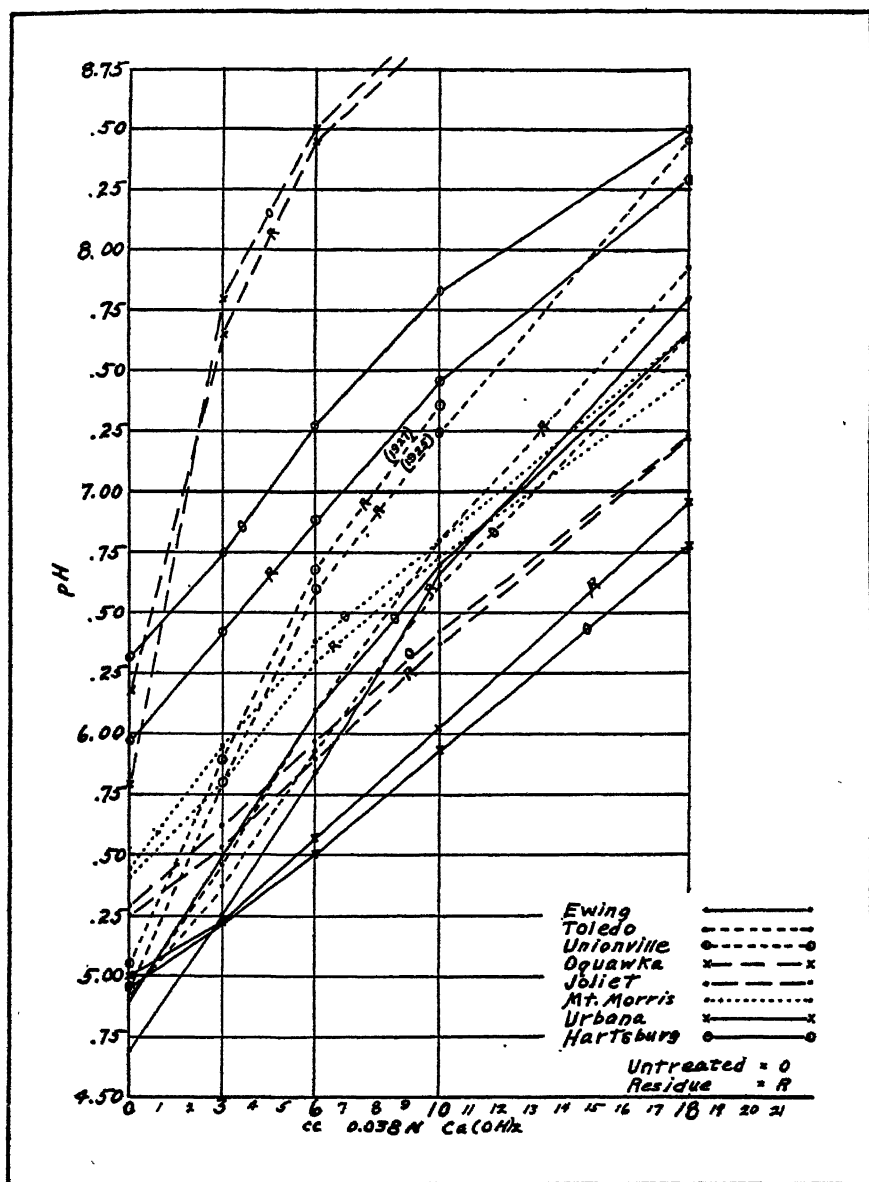
The results—obtained only on the surface samples of the unlimed soils—are to be shown in figure 2. As indicated by the steepness of the curves, the Oquawka sand possesses the least buffer power, and the Unionville, Ewing, and Toledo soils come next with a buffer capacity considerably greater than the sand but less than the remaining northern soils.

A method which gives results that are directly comparable is to estimate from figure 2 the amount of calcium hydroxide required to change the pH from one value to another. By eliminating Hartsburg and Oquawka, and extending the Urbana curve to 7.0, it is possible to compare directly six of the soils from pH 5.5 to 7.0. The data are given in table 1.

In order to include Hartsburg and Oquawka, we can use the values 6.3 and 7.5, with the subjoined data (table 2).

We see from table 1 that the Urbana soil is the most highly buffered of the

⁴Norton, E. A., and R. H. Bray. The Reactionary Soil Profile. To appear in *Jour. Amer. Soc. Agron.*

FIG. 2. BUFFER CURVES OF THE A₁ HORIZON

six, whereas Unionville has the least buffering power. In the case of table 2 Oquawka is least buffered, and Hartsburg is about the same as Toledo.

Lime-requirement.—The Hutchinson-MacLennan and the Hopkins lime-requirement methods paralleled the pH values in some cases, and in other cases they ran counter to those values.

Smith (29) working with Scottish soils, reports fair agreement between the results obtained by the Hutchinson-MacLennan method and the pH values. Crowther and Martin (11) say that the results obtained by the Hutchinson-MacLennan method are always lower than the equivalent amount of calcium hydroxide required by the electrometric titration method. They state further that the Hutchinson-MacLennan method is no indicator of the intensity of

TABLE 1
Amount of 0.038 N Ca(OH)₂ required to change the pH from 5.5 to 7.0

| SOIL | UNTREATED | RESIDUE | SOIL | UNTREATED | RESIDUE |
|------------------|-----------|---------|------------------|-----------|---------|
| | cc. | cc. | | cc. | cc. |
| Ewing | 9.6 | 8.1 | Joliet | 13.7 | 13.3 |
| Toledo | 9.3 | 8.3 | Mt. Morris | 11.6 | 12.2 |
| Unionville | 6.3 | 6.5 | Urbana | 14.1 | 13.0 |

TABLE 2
Amount of 0.038 N Ca(OH)₂ required to change the pH from 6.3 to 7.5

| SOIL | UNTREATED | RESIDUE | SOIL | UNTREATED | RESIDUE |
|------------------|-----------|---------|------------------|-----------|---------|
| | cc. | cc. | | cc. | cc. |
| Ewing | 9.3 | 7.7 | Mt. Morris | 11.2 | 12.1 |
| Toledo | 8.7 | 7.8 | Joliet | 11.6 | 11.5 |
| Unionville | 6.2 | 6.8 | Hartsburg | 7.8 | 8.2 |
| Oquawka | 1.8 | 2.4 | | | |

soil acidity but merely gives an indication of the amount of lime needed to reduce considerably the acidity of the soil.

On the other hand, Brioux and Pien (5) state that the Hutchinson-MacLennan method compares very favorably with electrometric titration to a pH of 8.0. Ogg and Dow (26) report close agreement between the pH values and the Hutchinson-MacLennan data in soils of similar colloidal content.

The data obtained in this study agree with those of the last two references. The figure which was obtained by the Hutchinson-MacLennan method representing the amount of calcium carbonate required by the soil was divided by the calcium carbonate equivalent of 1 cc. of standard calcium hydroxide used in determining the buffer action, giving the cubic centimeters of hydroxide that would be required to equal the first mentioned figure. By interpolation on the graphs then, it was found that the Hutchinson-MacLennan method compared

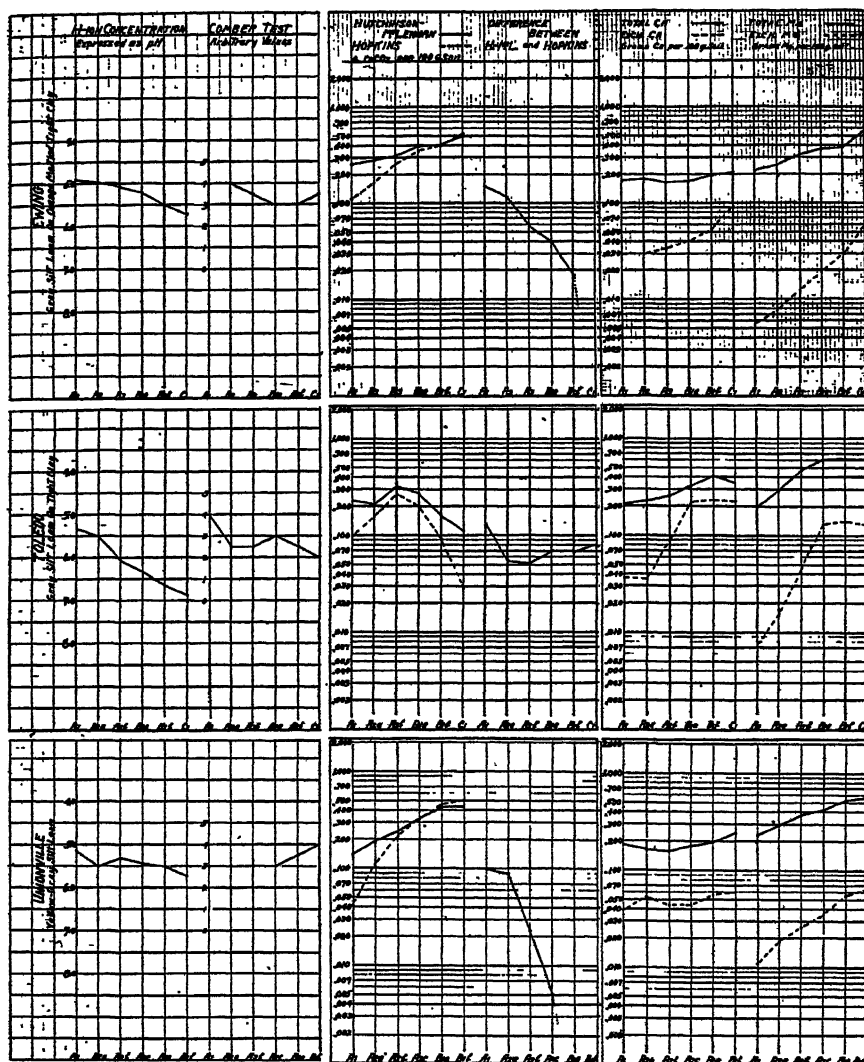


FIG. 3. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE EWING, TOLEDO AND UNIONVILLE FIELDS

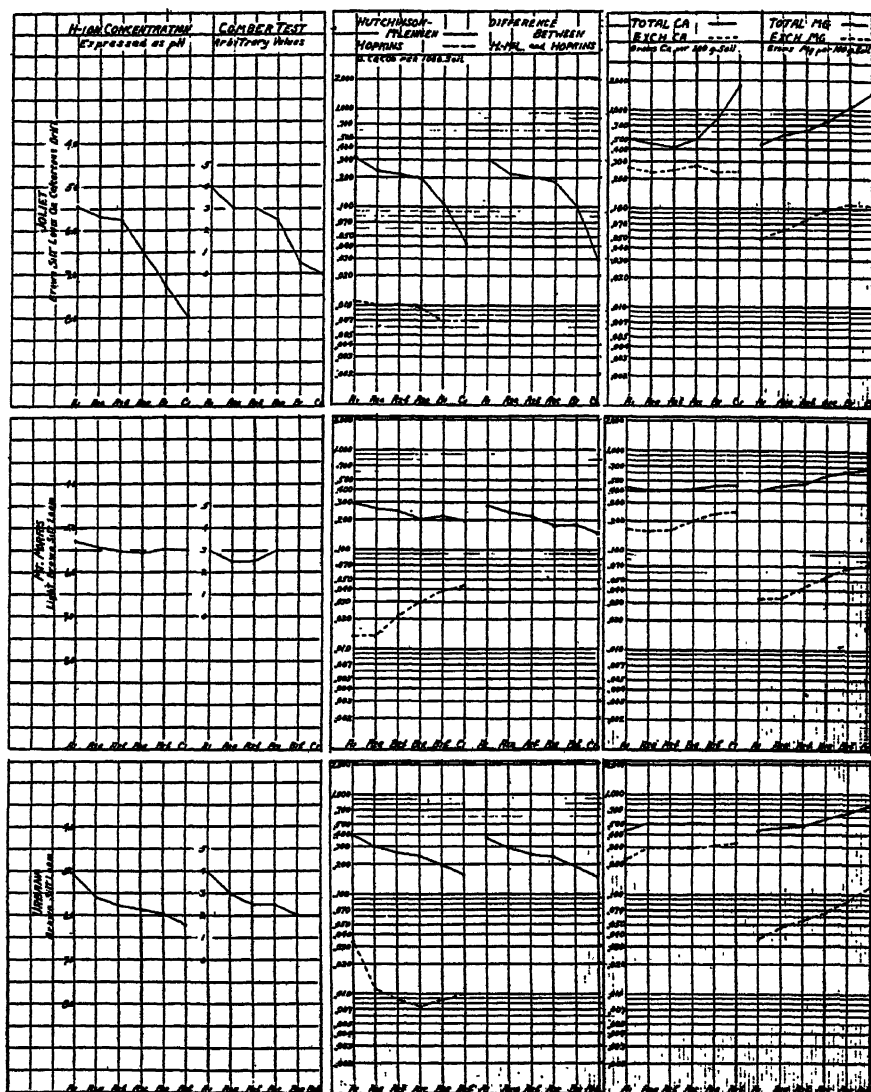


FIG. 4. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE JOLIET, MT. MORRIS, AND URBANA FIELDS

quite favorably with the electrometric titration to a pH of approximately 7.0. Taking the Ewing soil for example:

$$\frac{\text{H. — MacL. lime req. per 1,000,000}}{\text{CaCO}_3 \text{ equiv. of 1 cc. of } 0.038 \text{ } N \text{ Ca(OH)}_2} = \frac{2590}{190} = 13.6 \text{ cc.}$$

A line extended from 13.6 cc. on the graph in figure 2 intercepts the Ewing curve at pH 7.12. These data are shown in table 3.

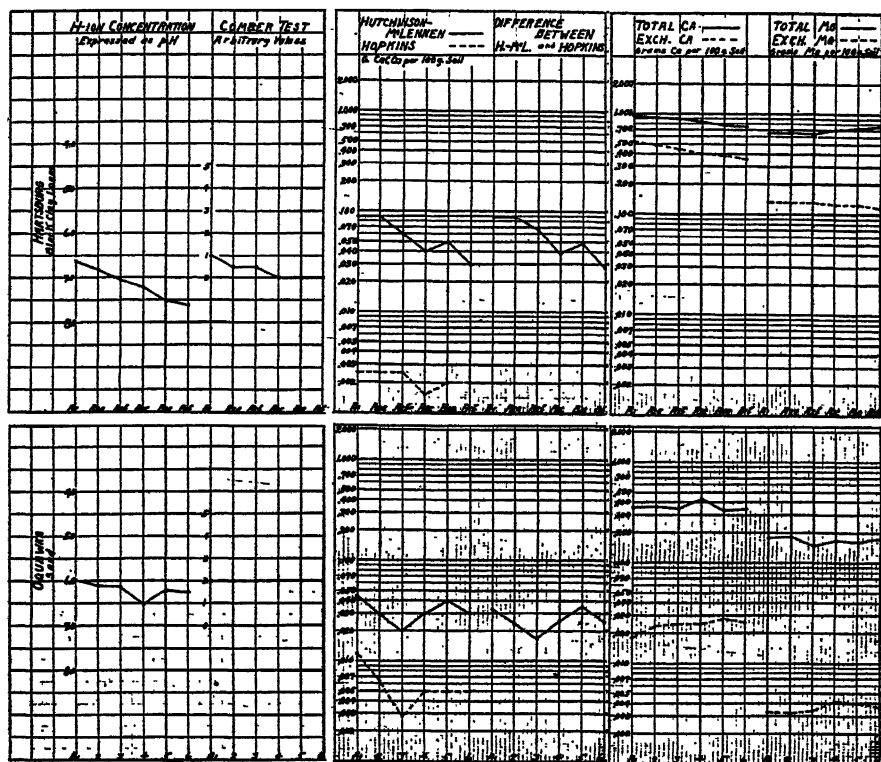


FIG. 5. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE HARTSBURG AND AQUAWKA FIELDS

Figures 3, 4, and 5 show that only in the soils of Southern Illinois—Ewing, Toledo, and Unionville,—do the lime-requirement values run counter to the pH; and in fact it is only in those soils that the lime-requirement increases with increase in depth. This latter statement is only partly true for the Toledo soil, since its points of high acidity as measured by both lime-requirement methods occur in layers A₂b and B₁a, or in other words at the juncture of the A and B horizons. There is no sign of such a “hump” in the curve representing pH. The curve shown under the caption, “Difference Between H-MacL. and Hopkins” is pretty largely the reverse of the lime-requirement curves.

At this point it is well to consider briefly the question of soil acidity as measured by different methods. Askinasi (2) classifies soil acidity as follows:

A. Active acidity,—in which a water extract of a soil contains free H ions; the acidity which is brought about by free acids or their acid salts.

B. Passive acidity,—in which the H ion is in the inactive, absorbed state. It is detectable by treatment of the soil with a salt solution.

(1) Unsaturation (exchange acidity),—in which the H ion is displaced by neutral salts.

(2) Hydrolytic acidity,—in which the H ion is displaced only by free alkalies or alkali-reacting salts.

TABLE 3

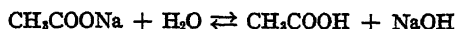
pH values obtained by interpolation. A comparison of Hutchinson-MacLennan lime requirement method with electrometric titration

| FIELD | TREATMENT | LIME REQUIRE- MENT BY H. MacL., (cc. Ca(OH) ₂) | pH |
|------------------|-----------|---|------|
| Ewing | O | 13.6 | 7.12 |
| | R | 12.8 | 7.06 |
| Toledo | O | 12.1 | 6.86 |
| | R | 11.6 | 7.02 |
| Unionville | R | 7.4 | 6.93 |
| | R | 8.9 | 7.06 |
| Oquawka | O | 2.4 | 7.37 |
| | R | 1.8 | 7.00 |
| Joliet | O | 16.5 | 7.08 |
| | R | 16.5 | 7.05 |
| Mt. Morris | O | 15.2 | 7.34 |
| | R | 16.5 | 7.33 |
| Urbana | O | 21.0 | 7.09 |
| | R | 20.0 | 7.18 |
| Hartsburg | O | 4.7 | 7.04 |
| | R | 7.4 | 7.08 |

Under B (1), Askinasi includes the methods of Gedroiz, Daikuhara, Hopkins, and others. All unsaturated soils he considers as belonging to one of three groups: 1. Soils which contain a large amount of active aluminum; 2. Soils which contain only a small amount of active aluminum; 3. Soils which contain no active aluminum. In the treatment of the soils of the first group with a neutral salt, the mineral acid resulting from the exchange process, e.g. $\text{Soil H} + \text{KCl} \rightleftharpoons \text{Soil K} + \text{HCl}$, is "neutralized" almost entirely by the Al. With the soils of the second group, only a part of the H ion is "neutralized" by the Al.

In this case, both H ion and Al ion are present in the filtrate. When the soils of the third group containing no aluminum are treated with neutral salts only the free mineral acid is found in the filtrate.

Hydrolytic acidity is characterized by the ability of the soil to neutralize the product of the hydrolysis of a salt that is composed of a strong base and a weak acid. For example, sodium acetate hydrolyzes as follows:



In an acid soil the Na ion replaces the adsorbed H ion of the soil and water is formed. The equilibrium of the above reaction is not established until all of the soil H ion has been displaced by the Na ion.

Therefore, the treatment of an acid soil with the salt of a strong base and a weak acid measures both exchangeable and hydrolytic acidity. For the ascertainment of the latter then, the results of a neutral salt extraction must be subtracted from the results secured by treatment with an alkali or an alkali-reacting salt such as sodium acetate.

In the Hutchinson-MacLennan method calcium bicarbonate is used rather than sodium acetate. The difference between the results obtained by the Hutchinson-MacLennan and the Hopkins methods, shown in figures 3, 4, and 5, therefore, represent essentially the hydrolytic acidity as defined by Askinasi (2) and others (7), (20).

It is seen that only in the soils from Ewing, Toledo, and Unionville does the hydrolytic acidity differ to any consequential degree from the total acidity as indicated by the calcium bicarbonate method. For only in these three soils does the exchange acidity approach closely or exceed the total acidity.⁵ Thus it can be said that these three soils are characterized by a comparatively high total acidity, and of this acidity a very large proportion is described as exchangeable, leaving a very small part as hydrolytic acidity.

Total calcium.—The relationships of total calcium to pH are not at all uniform. On the Mt. Morris field the curve for total calcium follows very closely that of the pH values; on the Hartsburg field there is less correlation between the two; whereas on the other fields it varies more widely. These results are similar to those reported by Némec and Gracanin (23).

Total magnesium.—As was the case with total calcium the correlation between total magnesium and pH is quite variable. The lower horizons of all types, excepting the Oquawka sand, contain more magnesium than do the surface layers. MacIntire (21) states that magnesium does not usually exist in humid soils as the carbonate, but is converted to silicates, whereas calcium is found partly as the carbonate and partly as the silicate, both of which are more readily leached than is magnesium silicate.

⁵ Naturally it is impossible for the exchangeable acidity to exceed the total acidity. Where such appears to be the case it merely indicates that the absolute values of the two methods cannot be compared directly. When compared relatively, however, the figures are significant.

In nearly every instance in the surface horizon, the percentage of total magnesium is approximately the same as the total calcium.

Exchangeable calcium and magnesium.—The exchangeable calcium content follows the total calcium for the various depths within each soil type, but in most instances the percentage increase or decrease in the exchangeable is greater than the corresponding change in the total. This statement holds true also for exchangeable magnesium in relation to total magnesium. The exchangeable calcium in the horizons B₁ and C₁ of the Joliet soil does not follow the total calcium content, as far as could be determined. As stated previously, the high content of carbonates interferes seriously with the accurate estimation of the replaceable bases of a soil and in the case of C₁ horizon of the Joliet soil this value was taken to be the same as that for B₁.

Page and Williams (27) reported that the amount of exchangeable calcium in a number of the soils which they studied was closely correlated with the pH values. With some Scottish soils Smith (29) found no real correlation in this respect except in soils of similar character.

TABLE 4
Exchangeable calcium and magnesium in surface (A₁) horizon expressed as percent of total calcium and magnesium respectively

| FIELD | Ca | Mg | FIELD | Ca | Mg |
|-----------------|------|-----|-----------------|------|------|
| Ewing..... | 17.1 | 2.5 | Joliet..... | 51.0 | 11.1 |
| Toledo..... | 17.4 | 3.7 | Mt. Morris..... | 39.2 | 8.2 |
| Unionville..... | 21.3 | 4.7 | Urbana..... | 51.0 | 8.0 |
| Oquawka..... | 5.4 | 1.7 | Hartsburg..... | 55.8 | 20.4 |

A very interesting point to observe is the difference in the proportion of total base which is in the exchangeable form in the various soils. This can be seen in the graphs in figures 3, 4, and 5. The data for the surface samples only are here presented.

It is readily seen that the northern soils fall into one group and the southern soils into a second group in this regard. The one exception is Oquawka sand which because of its low colloid content, falls into the southern rather than the northern group.

Catherwood and DeTurk (6) studying four soil types of Illinois—Light Gray Silt Loam On Tight Clay, Brown Gray Silt Loam On Tight Clay, Brown Silt Loam, and Black Clay Loam—found the percentage of total calcium present in exchangeable form to be least in the Light Gray Silt Loam, and increasing in the several types in the order given.

It is interesting to note that the pH of the A₁ horizon of all but the Hartsburg and Oquawka soils lies between 4.97 to 5.5. The total calcium of this layer of the three southern fields is found in the vicinity of 0.2 per cent, but in the three northern fields it lies between 0.4 and 0.5 per cent. Again, with the

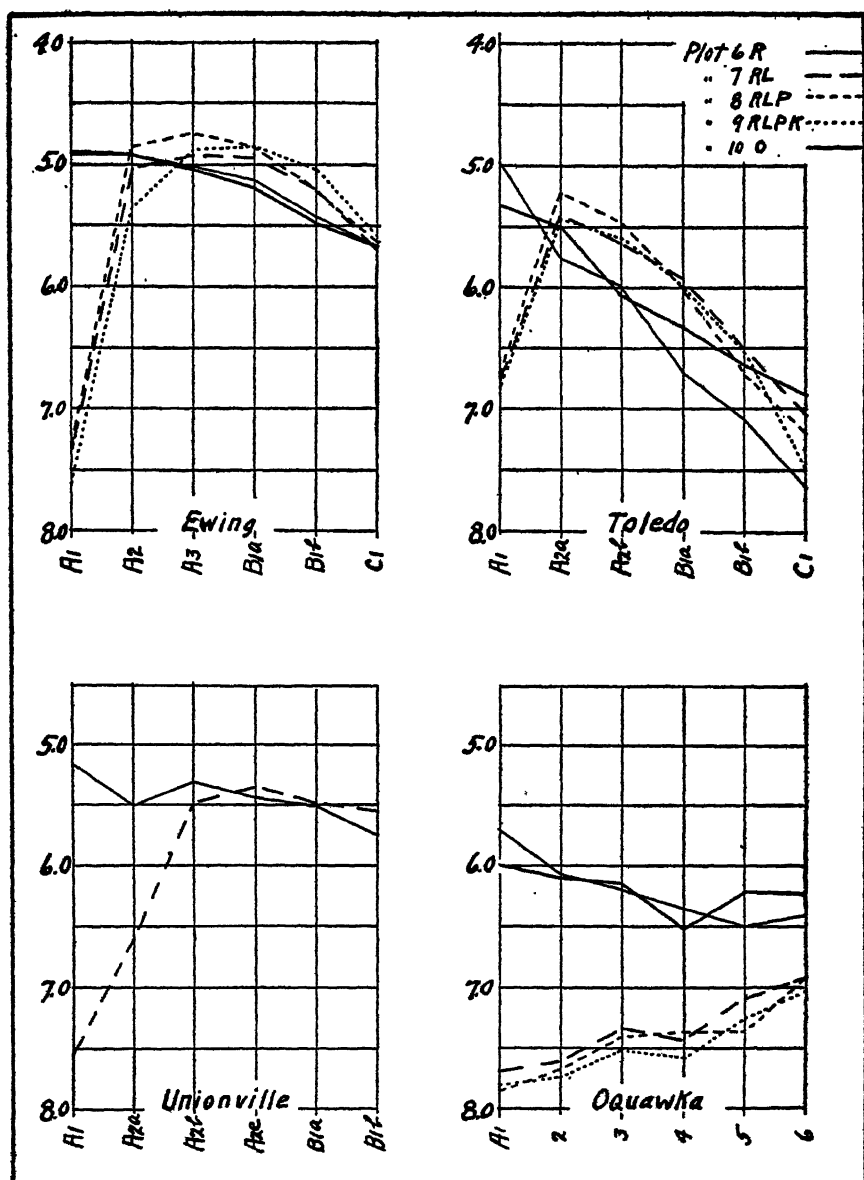


FIG. 6. EFFECT OF TREATMENTS ON H-ION CONCENTRATION (EXPRESSED AS pH) OF SOILS FROM FOUR FIELDS

same pH, the southern soils have an exchangeable calcium content within the limits of 0.026 and 0.038 per cent, whereas that of the northern soils is much higher, namely between 0.16 and 0.27 per cent.

In both the untreated and the residue plots there is no carbonate carbon in the upper layers of the Joliet soil nor in the entire profile of the other soils.

The high carbonate content of the subsoil on the Joliet field has little influence on the surface horizon of that soil, for one may observe the similarity in the chemical data of the A_1 horizons of the Joliet and Mt. Morris soils, although the Mt. Morris soil lacks carbonates and is quite uniformly acid in all horizons.

Comparison of soil treatments

The previous section dealt with a study of the untreated plots of the various soil types. The effects of soil treatments upon the soils in question as registered in the chemical studies will now be considered. The data for the treated as well as the untreated plots are shown in figures 6 to 17. The symbols used in these figures have the following significance:

O = No treatment

R = Non-legume crop residues, including wheat and oats straw, and corn stalks. Straw has been discontinued during the last four to five years. Legume catch crops are also included.

L = Limestone

P = Rock phosphate except on the east half of Urbana plots (marked E) where it represents steamed bone meal.

K = Kainit

Limestone has reduced the H-ion concentration of the surface layer in every case (figs. 6, 7, 8). Its effect upon the H-ion concentration has extended only through the A_1 horizon of the Ewing and Toledo soils; to the A_{2b} layer at Unionville, Joliet and Mt. Morris; to the A_{2c} layer at Urbana; to the B_{1a} layer at Hartsburg, and through all six layers at Oquawka. The data show somewhat lower pH values in the limed plots for the intermediary horizons of the Ewing and Toledo soils, but it would hardly be safe to ascribe these changes to the limestone treatments, especially when none of the data for lime-requirement or for total or exchangeable calcium and magnesium show corresponding changes in these horizons.

Crowther (9) studying the soils on the Rothamsted and Woburn fields found that on a medium acid soil which had received 3.8 tons of lime per acre for a period of 17 years the lime was responsible for an increase of 1.3 in pH, which from titration curves of one of the plots indicated a lime-requirement of two tons for the surface nine inches. He states that as a result of soil treatment the subsoil underwent changes similar to, but lesser in degree than those of the surface soil.

In his succeeding article (10), Crowther concludes in part,

The reactions of the unmanured and the limed and unlimed portions of the sulfate of ammonia plots on Rothamsted Park Grass and Woburn barley fields change steadily with

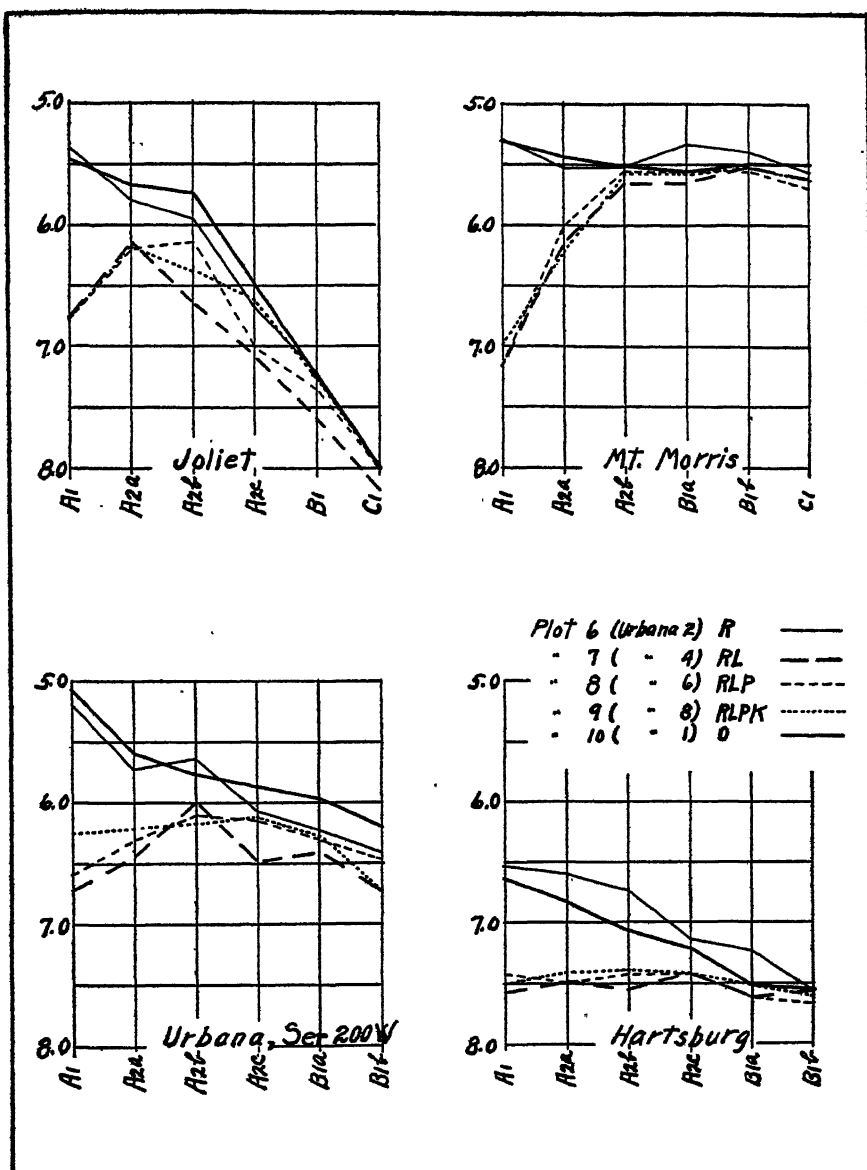


FIG. 7. EFFECT OF TREATMENTS ON H-ION CONCENTRATION (EXPRESSED AS pH) OF SOILS FROM FOUR FIELDS

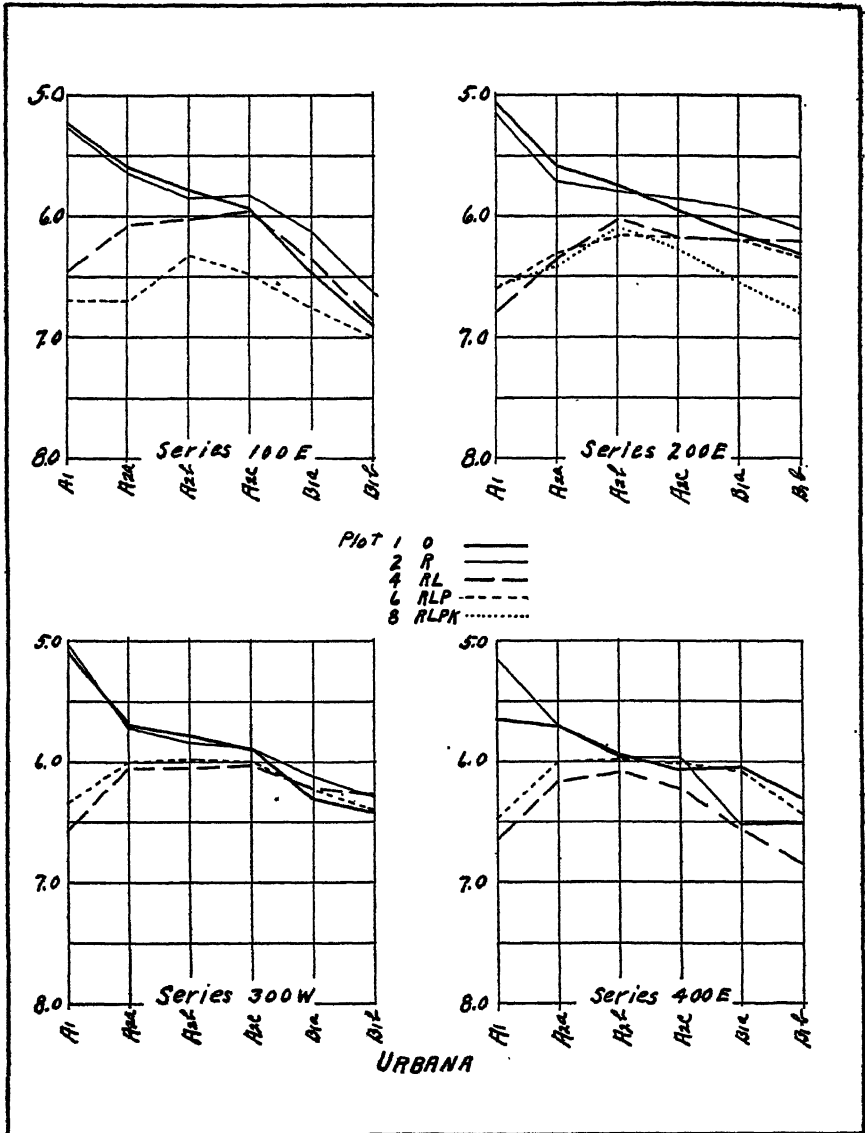


FIG. 8. EFFECT OF TREATMENTS ON H-ION CONCENTRATION (EXPRESSED AS pH)

For series 200W see figure 7

increasing depth, and at 36 inches still show the same relations as in the surface soil. The difference in pH values between the limed and unlimed portions of the Rothamsted soil is substantially constant at all depths down to 36 inches. The reaction of the subsoil plays an important part in determining the effect of liming.

The data which have been presented indicate that the response to liming of the Illinois soils studied differs markedly from the response observed by Crowther in the English soils.

The pH data for Urbana Series 200W are given in figure 7, and those for the remaining four series sampled at Urbana are to be found in figure 8. Comparison of the five series with one another shows similarity in the curves, and at the same time shows the soil variations. A striking similarity is to be observed between the variations in this soil as indicated by the H-ion concentration (fig. 8) and the variations registered by the Hutchinson-MacLennan lime-requirement test (fig. 12).

The influence of limestone is detected by this latter test to varying depths in the several soils. This influence, however, does not in all cases coincide with the pH data in this respect.

With the possible exception of the soil on the Ewing field, no effects of liming can be observed by the Hopkins test below A_1 .

Ames and Schollenberger (1) estimated the lime-requirement of a limed Wooster silt loam by 4-inch layers to a depth of 24 inches by the Hopkins, Veitch, and Vacuum methods. They found the effect of lime reaching to 24 inches or more. From results obtained by the Hopkins tests, Stewart (31) found that on Gray Silt Loam On Tight Clay of southern Illinois, limestone applied to the surface penetrated slowly into the second stratum (7 to 20 inches) but not at all into the lower sampling stratum (20 to 40 inches). His second stratum (7 to 20 inches) was not subdivided in sampling.

There is no conclusive evidence in the data here presented that soil treatments *in addition to liming* have had any influence upon the lime-requirement of these soils, or upon the amount of replaceable calcium and magnesium. In other words, the total effect of soil variation and of errors in sampling and in analysis is greater than any differences due to soil treatments other than liming.

In his work on Illinois soils, Catherwood⁶ was unable to detect any increase in exchangeable calcium in plots which had been treated with bone phosphate alone or in conjunction with potash; but when limestone was included in the treatment, bone phosphate showed a marked increase in exchangeable calcium over the limestone-without-phosphate plot.

In this connection can be mentioned the work of Smith (30) in which he sampled several soils before and after an interval of 12 months. Some differences were obtained in the exchangeable calcium content which he attributed to field error rather than to cropping.

The fact that, in this work on Illinois soils, liming seems to have influenced

⁶ Catherwood, M. P. Thesis, University of Illinois, 1927.

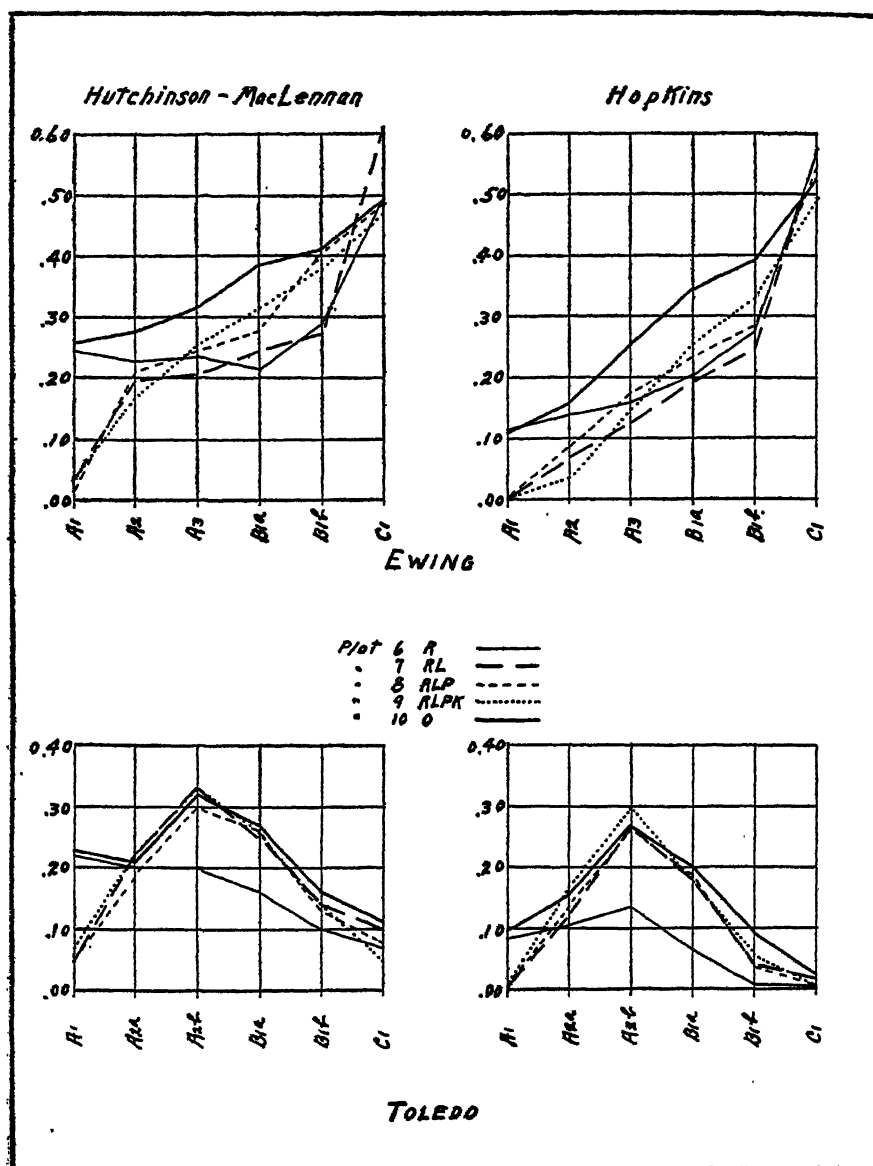


FIG. 9. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN AND THE HOPKINS METHODS IN GRAMS CaCO_3 PER 100 GM. SOIL.

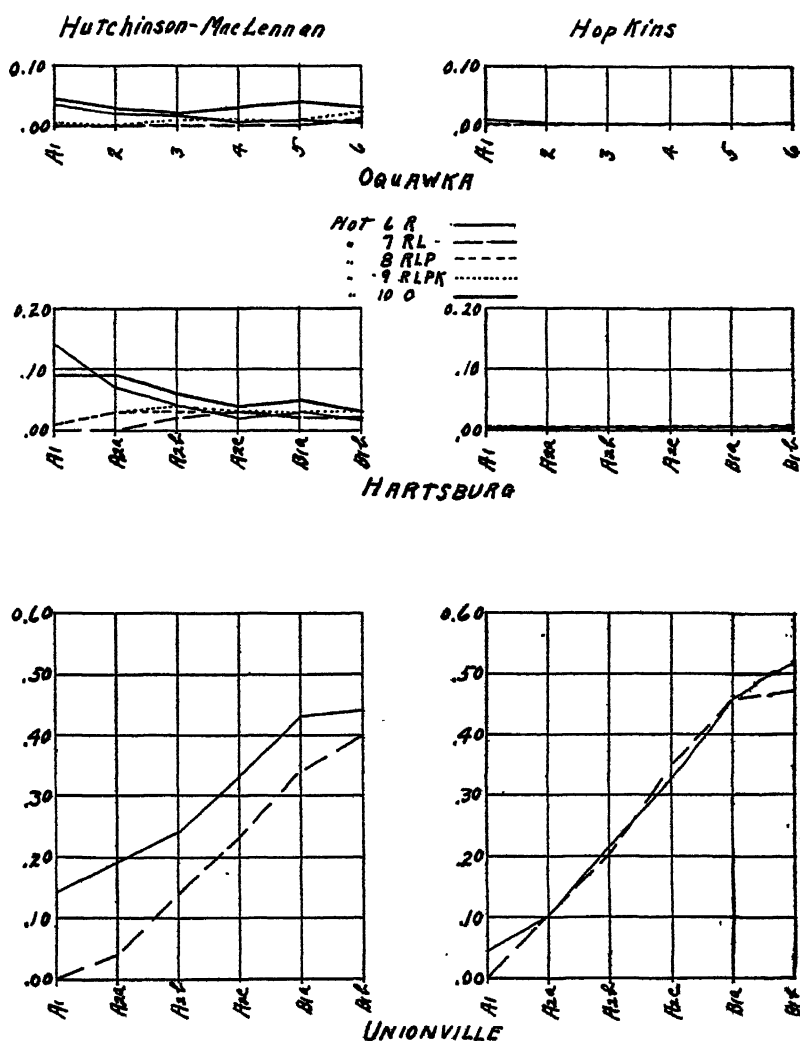


FIG. 10. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINGON-MACLENNAN AND THE HOPKINS METHODS, IN GRAMS CaCO_3 PER 100 GM. SOIL

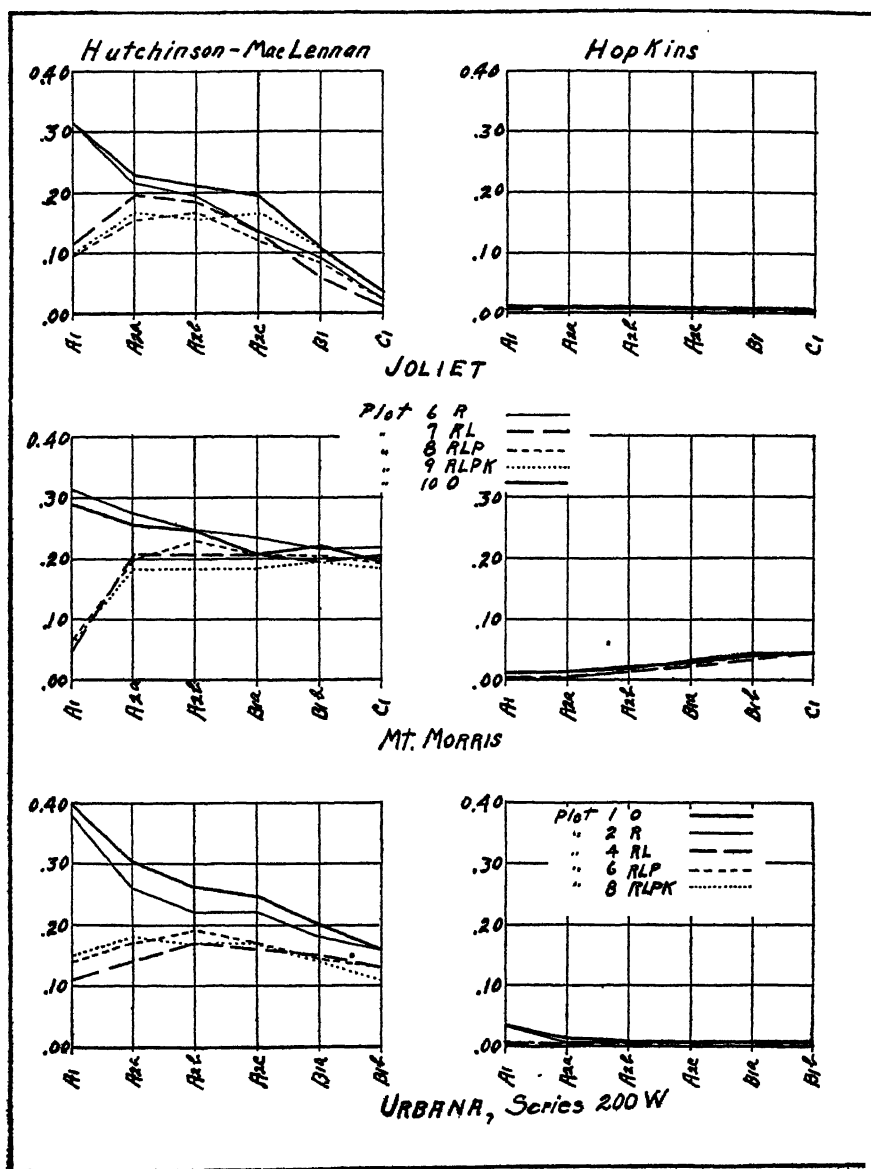


FIG. 11. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN AND THE HOPKINS METHODS, IN GRAMS CaCO_3 PER 100 GM. SOIL

the pH to a depth greater than it has affected either total or exchangeable calcium or the soil lime-requirement is probably indicative of the greater sensitivity of the H-ion determination.

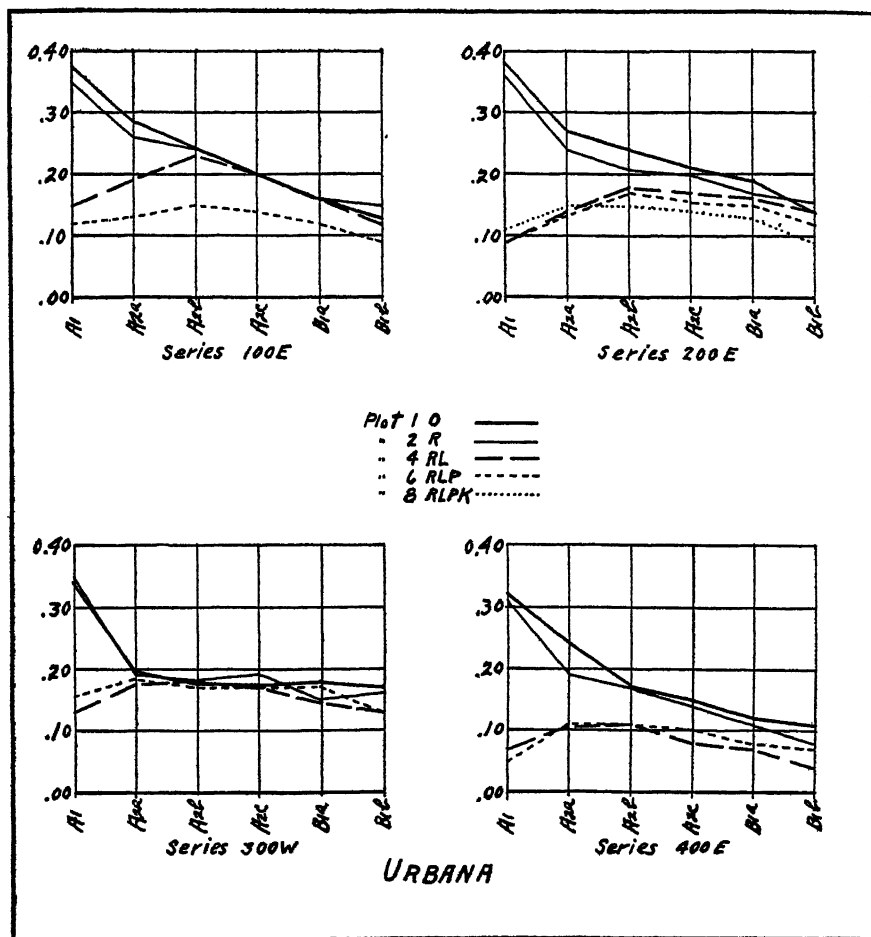


FIG. 12. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN METHOD, IN GRAMS CaCO_3 PER 100 GM. SOIL (DATA FOR HOPKINS METHOD NOT GRAPHED)

For series 200W see figure 11

In the case of total calcium, there is some indication that the calcium in the phosphate added is revealed in the analysis of the surface horizon. The increased amounts found in phosphated plots appear to be in excess of any differences attributable to soil variation, with the exception of the soil at Urbana. In the latter case one may observe in the field a gradual change in

type from Brown Silt Loam to Black Clay Loam as one passes from plots 1 to 10, although as mapped, the Black Clay Loam is confined to the south half of plot 8, and to plots 9 and 10. This soil variation undoubtedly accounts for a considerable portion of the greater calcium content in plots 6 and 8.

The work of Page and Williams (27), on the influence of various fertilizers upon the exchangeable base content of soils from the Broadbalk field, credits manure with having the most favorable influence upon the replaceable calcium oxide content of the surface soil. Treatment consisting of superphosphate, sodium sulfate, and ammonium sulfate came second in this respect, with superphosphate and ammonium sulfate third. Other treatments had less

TABLE 5

*Total yields of all crops except corn stover and straw of small grains for the 10-year period 1917-1926, in pounds of air-dry material per acre**

| FIELD | PLOT AND TREATMENT | | | | |
|--------------------------|--------------------|---------|----------|-----------|-----------|
| | 6 R | 7 RL | 8 RLP | 9 RLPK | 10 O |
| Ewing | 3,312 | 11,656 | 12,362 | 17,556 | 4,694 |
| Toledo | 5,944 | 13,257 | 14,150 | 16,412 | 6,148 |
| Unionville | 10,188 | 15,384 | 16,276 | 18,202 | 7,426 |
| Oquawka | 6,797 | 20,784 | 19,559 | 19,124 | 3,949 |
| Joliet | 18,657 | 19,631 | 24,603 | 26,611 | 16,466 |
| Mt. Morris | 21,700 | 31,034 | 32,798 | 34,665 | 19,569 |
| Hartsburg | 30,722 | 31,435 | 31,835 | 31,655 | 23,798 |
| | 1 O | 2 R | 4 RL | 6 RLP | 8 RLPK |
| Urbana Series 100E | 32,188 | 34,792 | 43,377 | 48,578 | 52,096 |
| Urbana Series 200W | 35,542 | 36,876 | 40,331 | 54,587 | 61,586 |
| Urbana Series 300W | 18,509 | 18,781 | 26,416 | 34,212 | 34,840 |
| Urbana Series 400E | 21,177 | 20,971 | 24,023 | 29,338 | 30,412 |

* The yields of the different fields are *not* comparable. One can only compare the treatments on each individual field, and, in the case of Urbana, on each individual series.

influence. It is not known to what extent natural soil variations played a part in these results.

Smith (30) treated soils representing nine different types with several salt solutions of 0.02 *N* and also 0.002 *N* concentrations. After treatment, the soils were washed thoroughly and the replaceable bases determined. The stronger of the two concentrations had a marked effect upon the absorbed base content of these soils. But the weaker solutions brought about only a very small change which, he stated, would be comparable to what might be expected from the application of soluble fertilizers in the field. Such alterations as could be observed within a period of several years would not, in his opinion, be greater than the natural variations occurring in the soil.

Relation of laboratory data to crop yields

Soil investigations, in addition to their scientific importance have added value if they bear directly or indirectly upon the crop producing capacity of the soil. It is for this reason that an effort has been made to determine the relation of crop yields to the chemical nature of the soil profile.

The combined air-dry yields of all crops in pounds per acre for the 10-year period 1917 to 1926 are given in table 5. Yields of corn stover and straw of small grains were not recorded for residue plots and hence are omitted from the table for all plots.

It is not possible to compare the total yields on one field with those of any other since no two fields were cropped exactly alike. Even if the rotations were identical it would be only by chance that the same crop was grown concurrently on two or more fields.

TABLE 6
Correlations between crop yields and chemical analysis

| | HORIZON | NORTHERN FIELDS | SOUTHERN FIELDS* |
|----------------------------------|----------------------------------|-----------------|------------------|
| Total Ca..... | A ₁ | 0.266 ±0.140 | 0.836 ±0.049 |
| | A ₁ + A _{2a} | 0.156 ±0.147 | 0.664 ±0.091 |
| Exchangeable Ca..... | A ₁ | 0.1006 ±0.149 | 0.474 ±0.127 |
| | A ₁ + A _{2a} | 0.139 ±0.148 | 0.502 ±0.122 |
| H. — MacL. lime requirement..... | A ₁ | -0.179 ±0.145 | -0.751 ±0.071 |
| pH..... | A ₁ | 0.213 ±0.144 | 0.898 ±0.032 |

* Oquawka field included in this group.

The following weights in pounds per bushel were used in these calculations: wheat, 60; oats, 32; corn, 70; clover seed, 60; soybeans, 60; cowpeas, 60; rye, 56.

Correlations were made of crop yields with total calcium, with exchangeable calcium, with the Hutchinson-MacLennan lime-requirement results, and with the pH values, the short method recommended by Phillips (27a), being used. The data are given in table 6.

It will be seen that in the northern fields the correlations are not significant. In each case the probable error is quite large. This indicates that variations in the crop producing power of those soils are dependent upon factors other than the presence of available calcium and the absence of acidity. In other words, the soil properties measured in this study are not critical factors as related to crop production on these soils.

On the other hand, in the soils of the southern fields the correlations of crop yields with calcium and with acidity are close, particularly in the case of total calcium and of pH values. These factors are of great importance to crop

production on these soils. In field practice it is recognized that the addition of lime is usually the first and principal step to be taken in increasing the yields of the southern soils. This statement applies equally well to the Oquawka sand.

It will be observed that in the southern group the correlation of yield with exchangeable calcium is considerably lower than with total calcium. This fact can be readily explained. Liming of the soils of this group has invariably produced a larger percentage increase in exchangeable calcium than in total calcium. However, in the case of the Oquawka sand, which is included in this group, the absolute increase in exchangeable calcium has been very small in contrast to the other three soils, although the gains in crop yields have been large. Thus, the inclusion of the sand has markedly lowered the correlation coefficient. If the Oquawka sand is omitted, the correlation of crop yields with exchangeable calcium in the remaining three fields of this group is even closer than it is with total calcium, namely $.887 \pm .041$.

Mention should be made of the correlations of yields with the total and exchangeable calcium of the first two horizons, A_1 and A_{2a} , combined. When this is done the correlation of yields and total calcium is lowered in both northern and southern groups. On the other hand, the correlation with *exchangeable* calcium has been raised. These facts emphasize the greater penetration of exchangeable calcium as compared to the non-exchangeable portion, and also indicate the importance of exchangeable calcium in plant growth.

No effort has been made to account quantitatively for the calcium of the lime-stone that has been applied to these soils since neither the original calcium content nor the amount removed by the crops is known.

A rather important observation which has already been alluded to (p. 154) is, that in spite of the differences noted in the soils and in the crop yields between the southern and the northern fields, the pH of the surface horizon of the soils of both groups is approximately the same, omitting Hartsburg and Oquawka. The application of limestone has decreased the exchangeable as well as the active acidity and increased the total and exchangeable calcium content. As a result the H-ion concentration has been lowered, and in the case of the southern group, the crop yield greatly augmented.

These facts indicate that the H-ion concentration of these soils may not be a primary factor in crop production. The instance of the Oquawka sand with its very low yields and yet comparatively high pH substantiates the above presumption. Odén (25) states that, in general, the pH value at which a distinct decrease in yield occurs is probably a function of temperature and climate.

With the soils in question low base content and high exchangeable acidity due to the climate and soil age have resulted in a pH value that is accompanied by a distinct decrease in yield. In the north a somewhat differing climate and age of the soils have resulted in approximately the same pH value as above,

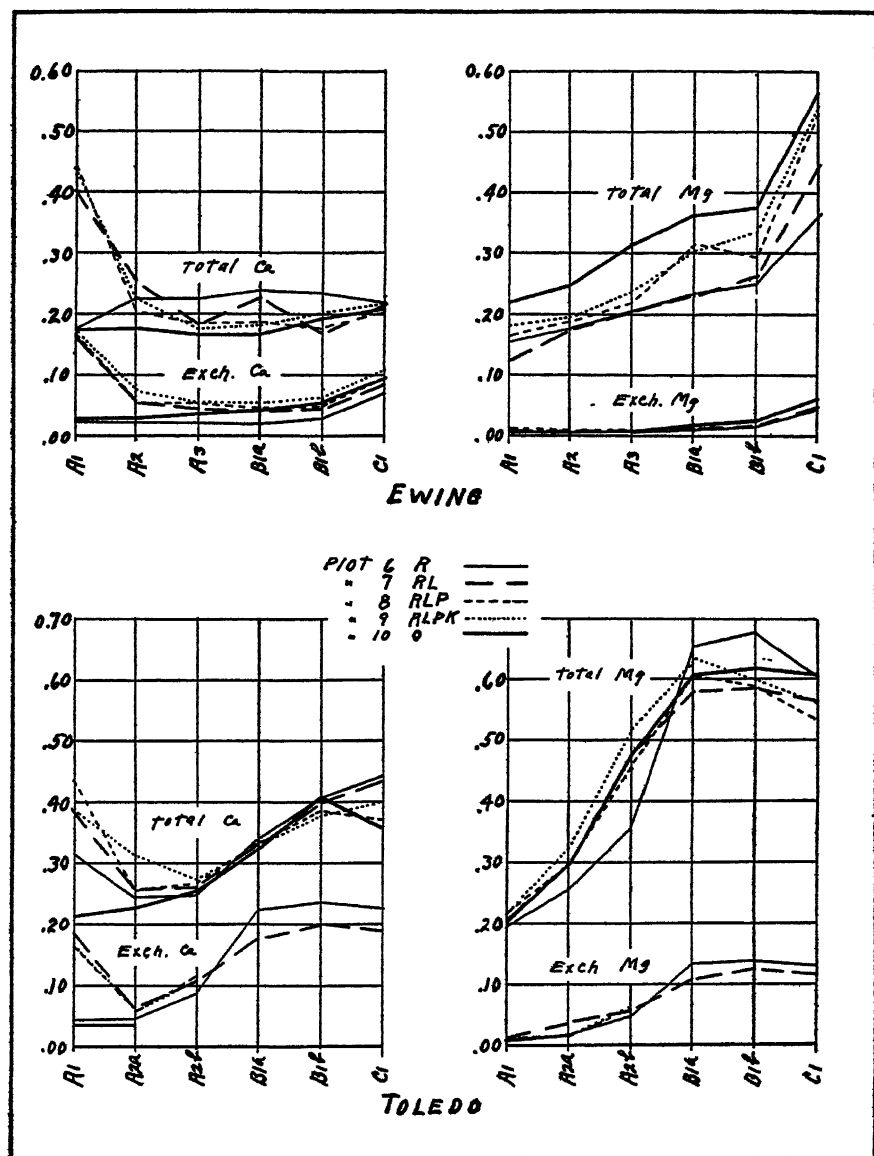


FIG. 13. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

but with a comparatively high base content, low exchangeable acidity, and high yields.

DISCUSSION

The results which have been presented in this investigation are indicative of the weathering processes that have taken place in the field. As a result of three principal factors—greater age, higher rainfall, and milder winters—the soils of the southern fields have undergone a more thorough removal of the basic constituents from the upper layers than have the soils of the north half of the state.

The Ewing and Unionville soils are characterized as having a lime requirement which increases greatly with depth, and the major portion of this acidity falls under the classification of exchangeable acidity. Hydrogen ions play an important part in the adsorption complex of these soils, particularly in the subsoil. In other words, these soils are quite unsaturated with respect to alkaline earth ions. There is a remarkable similarity in the chemical analysis of these two soils (*see* fig. 3), although their profile descriptions differ considerably. The Unionville soil is the only one used in this study which is located in an unglaciated region.

The soil from the Toledo field is in the same category as the Ewing and Unionville soils with respect to calcium and magnesium content, although its acidity as measured by the lime requirement methods does not increase steadily with depth as is the case with the latter soils. The type—Gray Silt Loam On Tight Clay—is characteristically variable, with the frequent occurrence of "slick spots."

The proportions of total calcium and magnesium that are in the adsorbed state are low in these southern soils, but the use of limestone on the treated plots has brought this proportion up to a figure quite comparable to that of the unlimed northern soils.

Crop yields on the untreated plots of the southern fields are usually low and are greatly enhanced by the use of limestone. This fact is well brought out in the correlation of yields with the chemical analyses. In every case the correlations are significant.

On the other hand, the soils of the northern fields are more highly buffered; they contain a greater amount of both total and exchangeable bases; and the proportion of total base that is exchangeable is much higher than is the case with the southern fields. The lime-requirement by the calcium bicarbonate method is fairly high in the surface layer, but it decreases with depth. Most of the acidity in these soils is hydrolytic rather than exchangeable.

Apparently this hydrolytic acidity is not injurious to plants. According to Gehring (15) a certain amount of exchangeable acidity in a *zeolitic-rich* soil caused no plant injury, but the same amount of acidity in a *zeolitic-poor* soil resulted in diminished plant growth. Haastert (16) likewise states that it is the exchangeable acidity which is responsible for plant injury.

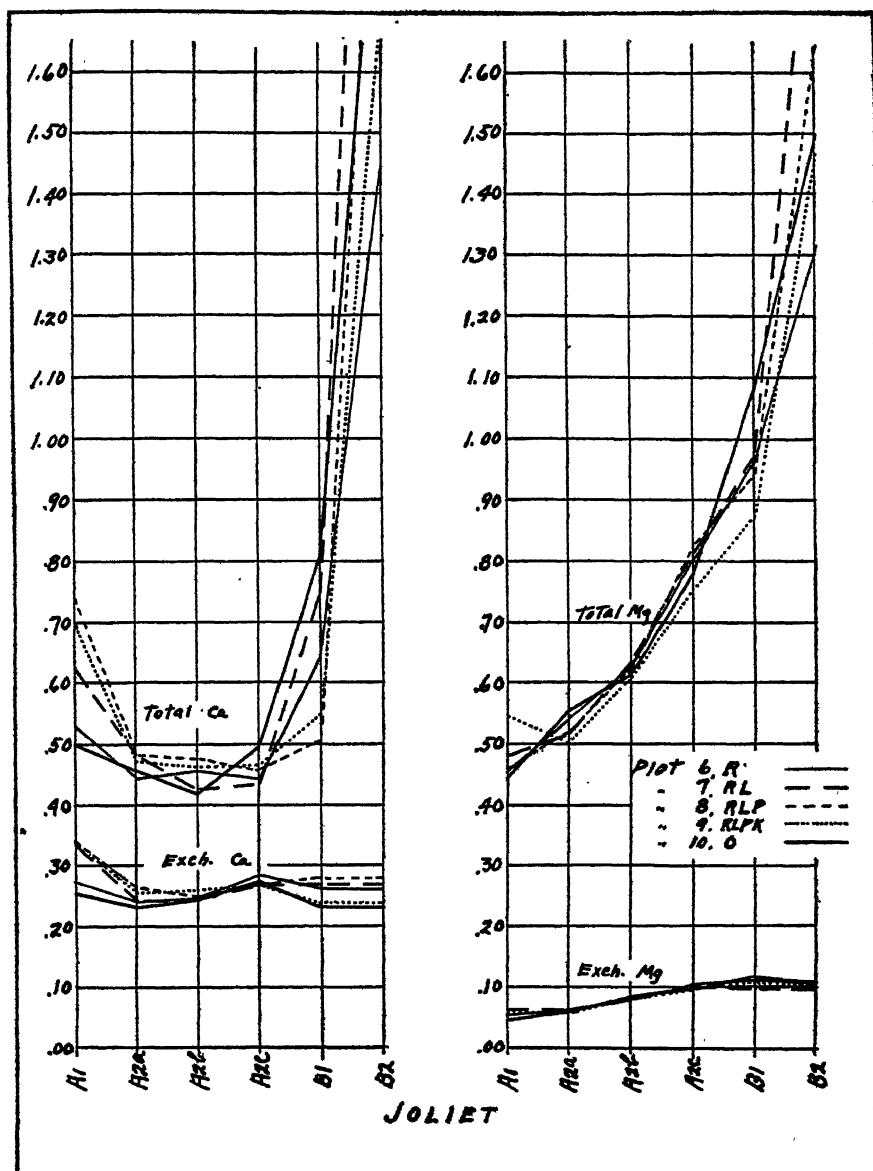


FIG. 14. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

The Oquawka field is located in the northern part of the state, but the yields on the untreated soil are low. This sand contains almost as much calcium as does the soil on the Mt. Morris field, but the amount that is exchangeable is decidedly low in the former. The poor buffer action and the low percentage of exchangeable bases is due, of course, to the paucity of alumino-silicate complexes capable of adsorbing basic ions. The crop yields on the untreated plots of the Oquawka field are lower than one would expect from observation of the pH values and the acidity as measured by the lime-requirement methods. Therefore, it is quite probable that available calcium or calcium and magnesium are the chief limiting factors in this sand.

It is hardly necessary to refer to the Black Clay Loam at Hartsburg with its high crop yields which respond but little to soil treatment. This soil is characterized by a low acidity and a relatively high total and exchangeable calcium and magnesium content in all horizons, with the greatest amount of the set materials, excepting total magnesium, in the surface layer. The exchangeable calcium in the A₁ horizon of the unlimed plots constitutes a somewhat larger proportion of the total calcium than in the plots that have received limestone. In other words, the total calcium content—and also the carbonate calcium—was raised by the addition of limestone without increasing the exchangeable calcium to any appreciable degree. Thus, while this soil does not appear to be highly buffered (*see* table 2), this is probably because its high buffer capacity is already nearly satisfied by the high content of native soil bases.

SUMMARY

Soils from treated and untreated plots on eight Illinois experiment fields representing as many soil types were sampled by 3- to 4-inch strata. These soils were subjected to the following tests and analyses: Hydrogen-ion concentration, Comber KCNS test, buffer action, Hutchinson-MacLennan and Hopkins lime-requirement tests, total and exchangeable calcium and magnesium.

Several different solutions for the replacement of calcium and magnesium were tried, with the final adoption of ammonium acetate.

In all cases the pH values increased with depth, the increase being greatest in the Joliet soil whose subsoil is highly calcareous, and least in the soils from the Mt. Morris and Oquawka fields.

The Comber test agrees closely with the pH values.

The Oquawka sand was the most poorly buffered, whereas the Urbana, Joliet, and Mt. Morris soils possess the greatest buffer capacity.

The Hutchinson-MacLennan lime requirement data compare favorably with electrometric titration with calcium hydroxide to a pH of approximately 7.0.

Only in the southern soils does the lime-requirement as measured by the Hutchinson-MacLennan test increase with depth. These soils are characterized by a comparatively high total acidity, of which a very large proportion

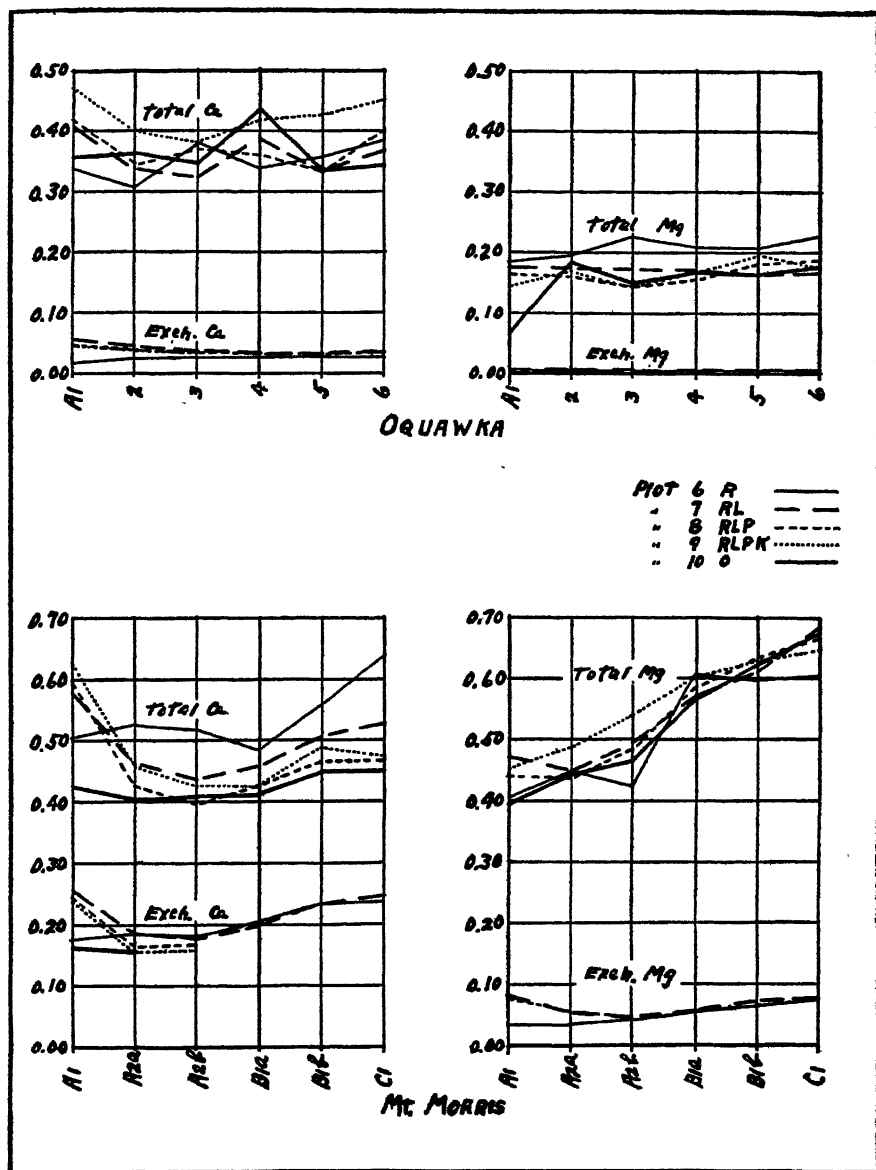


FIG. 15. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

is the so-called exchangeable acidity, and only a small part is hydrolytic acidity.

In a very general way the pH values vary with the total calcium content, with the exception of the Black Clay Loam at Hartsburg. This soil being a

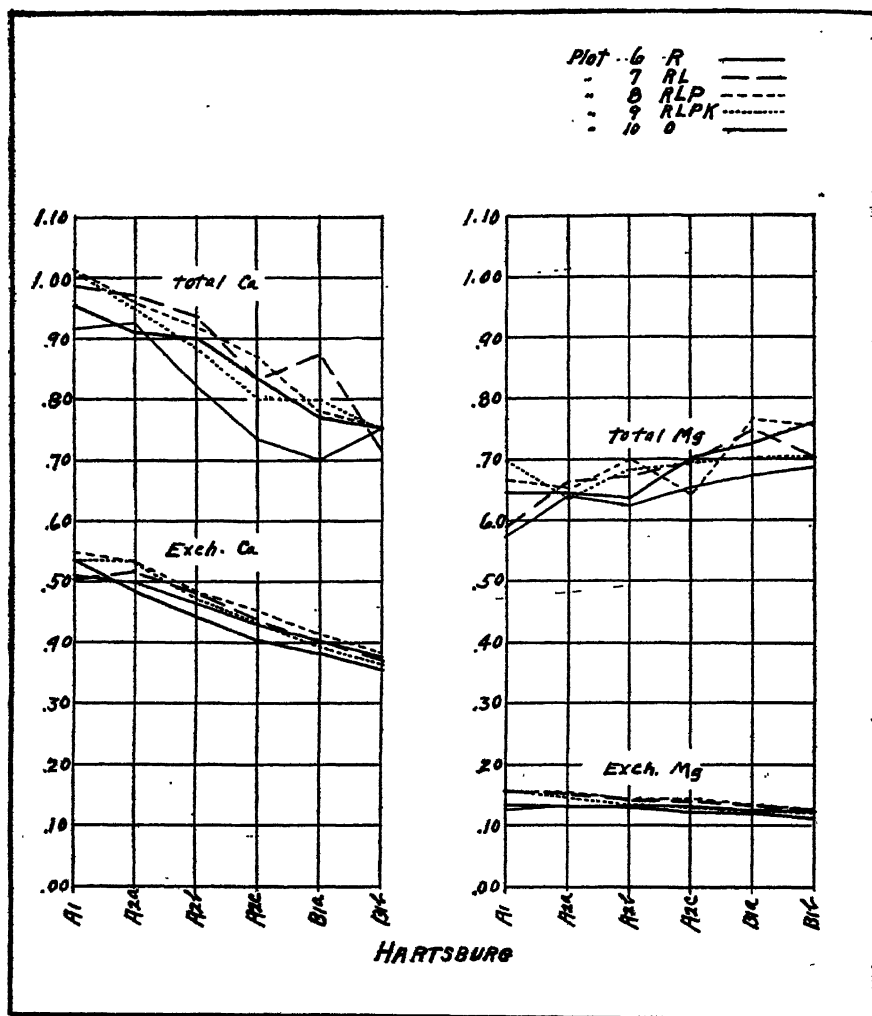


FIG. 16. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

comparatively immature one, its calcium has not been leached out of the surface to the extent that it has in the older soils.

In most instances the total magnesium content in the surface horizon is approximately equal in amount to the calcium content in that horizon, but

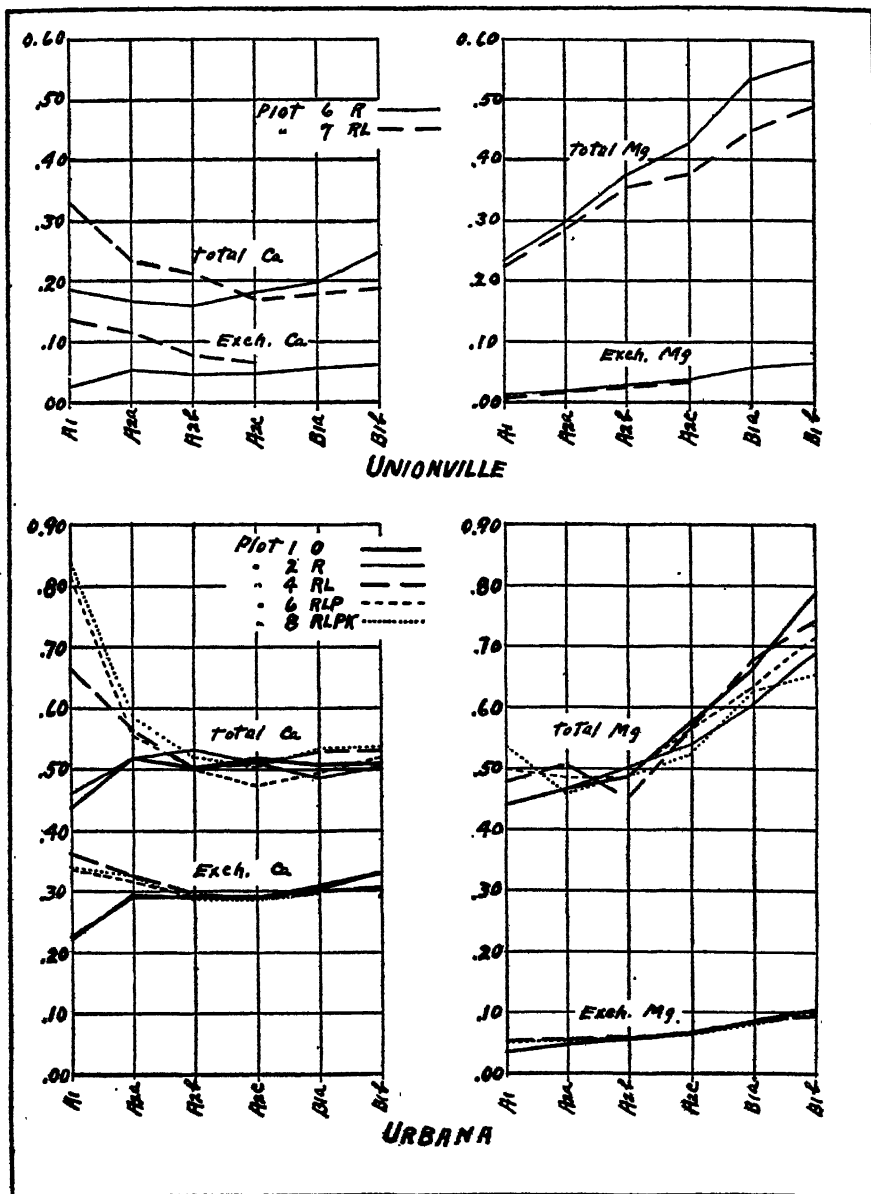


FIG. 17. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

in the subsoils there has been a greater accumulation of magnesium than of calcium.

The curves for exchangeable calcium and magnesium follow the curves for total calcium and magnesium respectively, but the percentage of change with depth is greater with the exchangeable fraction than with the total amounts of these bases.

In the upper layers exchangesable calcium and magnesium constitute a much greater portion of the total amounts of these elements in the soils from Joliet, Mt. Morris, Urbana, and Hartsburg than is the case with the Oquawka sand and the three southern fields. Undoubtedly this is the result of the more thorough leaching that has taken place in the southern three fields due to greater age of the soils, higher rainfall, and milder winters, and in the Oquawka field due to the permeable character of the sand.

The application of limestone has reduced the H-ion concentration and the lime-requirement, and increased the total and exchangeable calcium content of the surface layer in every case.

The influence of limestone penetrated to different depths in the various soils, but only in one soil, Oquawka, was it observed below the A horizon.

This influence could be detected more readily by the pH determination than by any of the other tests.

Natural soil variations were greater than any measurable influence of treatments other than liming.

The coefficient of correlation of crop yields with total and exchangeable calcium and with acidity of the A₁ horizon is very high in the southern soils, but low in the soils from the northern fields.

The hydrogen-ion concentration of the A₁ horizon of the southern fields and three of the five northern fields was approximately identical although crop yields were considerably greater in the northern fields. The data at hand indicate that the H-ion concentration may not be a primary factor in crop production so far as its direct effect on crop growth is concerned.

In the soils of Ewing, Toledo, and Unionville, the chief chemical factors which limit crop growth are probably the presence of exchangeable acidity and the low percentage of exchangeable bases. In the Oquawka sand, an insufficient amount of available calcium appears to be the main obstacle in the way of high crop yields. With respect to the Joliet, Mt. Morris, Urbana, and Hartsburg soils, none of the factors studied can be considered as limiting.

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FIRST SUBCOMMISSION OF THE FIFTH COMMISSION, INTERNATIONAL SOCIETY OF SOIL SCIENCE MEETS AT DANZIG

HUGO CARL

Danzig

The meeting of the First Subcommittee of the Fifth Commission of the International Society of Soil Science held in Danzig, May 20, was devoted to a conference about the great soil map of Europe and the problem of "Braunerde." About 50 soil scientists, representing 18 countries were present.

Dr. Stremme, president of the commission for the soil map of Europe, gave the results of his work on the map.

The maps of Russia, Poland, Czechoslovakia, Latvia, Esthonia, and Hungary are as complete as some regions of Germany. It is expected that the meeting of Leningrad in 1930 will find nine-tenths of the map finished. The soil maps of South America and the nearly complete ones of Asia that were exhibited, clearly indicated that the idea of continental soil maps is arousing interest outside Europe.

The interest of the countries was indicated in a report of Dr. Rothkegel of Berlin on the demands for the new valuation of arable land. His report stated that only through soil science is it possible to get exact and comparable methods of assessing the landed states.

Dr. Till of Wien gave details of the organization for tracing agricultural soil maps in Austria. The organization, drawn up with the help of the chambers of agriculture, is working efficiently, is cheap, and self supporting.

Prof. del Villar of Madrid gave a special report on the different soil types of Spain. The report was accompanied by a number of photos of profiles and of different types of vegetation.

The problem of "Braunerde" was treated in detail by Dr. Prassolov of Leningrad, Prof. Stebutt of Belgrade, K. Schlacht of Ludwigshafen, and Sellke of Hannover.

Schlacht presented an excellent method for the conservation of soil profiles: a piece of cardboard is covered with a special lacquer made by I. G. Farben of Ludwigshafen and is pressed for five minutes against the profile, which is thereby durably fixed.

The reports were effectively illustrated by a 3-day excursion into the districts of the Free City of Danzig.

The general discussion resulted in three resolutions being passed by the assembly: 1. A subcommission shall be appointed for the study of the coloration and methods of tracing for the soil map of Europe.

2. The types of soil around the Mediterranean Sea shall be studied by a subcommission of scientists from the bordering countries.

3. All sorts of profiles of "Braunerde" from all countries, preserved by the Schlacht or other methods shall be collected and sent to Prof. Stremme. After the study of the different types, Prof. Stremme shall give new directions for the classification and characterization of "Braunerde."

The whole meeting gave evidence of the desire on the part of all countries to perform international scientific services for the benefit of all.

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: I

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The mineral fraction of the soil colloidal material which constitutes the residue of rock weathering is composed mainly of the hydrated oxides of silicon, aluminum, and iron together with certain proportions of alkaline and alkaline earth bases. This material exists in the soil normally in the form of a gel which is mixed with, and coats the coarser particles of silt, sand, and gravel. The quality and quantity of this gel has a profound influence upon the structure of the soil, its workability, and its waterholding power; upon the capillary and gravity movement of water; and upon the adsorptive and exchange capacity of the soil and its buffer action.

The composition of the colloidal material varies greatly and depends apparently upon genetic forces and climatic conditions, such as temperature and precipitation, rather than upon the composition of the parent rock (17). The following outstanding facts from the work of Robinson and Holmes (22) are of interest in this connection.

The higher the silica/sesquioxide ratio the higher the proportion of the mono- and divalent bases. The lower this ratio, that is, the higher the proportion of sesquioxides, the smaller the quantities of bases present. In general the high-ratio colloids are found in arid regions and the colloids with a low ratio occur chiefly in warm, humid regions.

The colloidal behavior varies greatly with the composition. Swelling, viscosity, dispersibility, heat of wetting, adsorption of bases and basic dyes, and base exchange are all manifested to a greater degree by the colloidal materials with a high silica/sesquioxide ratio than the materials in which this ratio is low (2, 13, 15). The materials with a high ratio are also more electronegative, as measured by the quantities of a basic dye or of a solution of $AlCl_3$ necessary to neutralize the negative charge of the particles (13, 18). Particles having a high proportion of silica and bases remain electro-negative in acid as well as in neutral and alkaline solutions, whereas a high proportion of sesquioxides leads to an amphoteric behavior, the particles becoming electro-positive in acid solutions (13). The author has further shown that in agreement with this behavior the materials high in sesquioxides adsorb appreciable quantities of anions such as Cl and SO_4 from acid but not from neutral solu-

tion. The behavior of a given soil material has been shown by Gedroiz (8) to be influenced by the nature of the exchangeable cations present.

The author has recently established a quantitative relationship between the points of maximum swelling and the base exchange capacity in the case Na-saturated soil colloids (15). It was also shown that a gradual increase of Na and a corresponding decrease of Ca (the sum of the two ions being kept constant and equal to the exchange capacity) increased the negative charge, the dispersibility, and the swelling to a maximum. The enormous swelling of certain bentonites which is ascribed to a micaceous structure ["one dimensional colloid" (1)] was shown to be due to the nature of the exchangeable cations. When electrodyalized, the bentonite lost its abnormal power to swell but regained this power after saturation with NaOH.

NEGATIVE ADSORPTION

In a study of the adsorption of anions by soil colloids of varying silica/sesquioxide ratio, the adsorption of the Cl and SO₄ ions was always found to

TABLE 1
Negative adsorption of the Cl ion by Na- K- Ca- and Ba-saturated Sharkey soil colloid

| TREATMENT | CONCENTRATION OF Cl IONS | | DIFFER- ENCE | ELECTRICAL MIGRATION |
|-------------------|-----------------------------|-------------------------|-----------------|--|
| | Original solution | Superna- tant liquid | | |
| | <i>N</i> | <i>N</i> | <i>N</i> | $\frac{\mu}{\text{sec.}}$ $\frac{1 \text{ volt/cm.}}$ |
| Na-saturated..... | 0.2002 | 0.2095 | 0.0093 | -3.1 |
| K-saturated..... | 0.2004 | 0.2058 | 0.0054 | -1.7 |
| Ca-saturated..... | 0.1979 | 0.2010 | 0.0031 | -0.6 |
| Ba-saturated..... | 0.1998 | 0.2020 | 0.0022 | -0.4 |

be negative in neutral and alkaline solutions. The magnitude of this negative adsorption was then measured. Five-gram samples of Na-, K-, Ca- and Ba-saturated Sharkey soil colloid were shaken each with 50 cc. 0.2 *N* solution of the respective chloride. The Cl ion was determined in the supernatant liquid after standing 3 days. The results are given in table 1. [For details see original paper (16).] The last column gives the cataphoretic movement of the particles.

The experiment shows that the different cations cause a negative adsorption of the Cl ion which varies in the same order as was previously found in the case of swelling and dispersibility. One is at once reminded of the work of Procter and Wilson (20, 21) and especially of the work of Loeb (12) on the colloidal behavior of the proteins. If a definite relationship between the negative adsorption and the colloidal behavior of soil materials could be established, the problem would be placed on a quantitative basis. We shall see to what extent this is possible.

The fact that the charge, as measured by cataphoresis, is high for the Na-saturated particles indicates that this ion dissociates

from the surface of the particles while the Ba ion dissociates the least. The electronegative soil particle or micelle is therefore surrounded by a swarm or atmosphere of cations. This, the so-called outer layer of ions in the Helmholtz double layer, is not to be looked upon as a layer in the form of a concentric shell, but rather as being diffuse, as suggested by Gouy (9). The distribution of the ions in the outer layer must be similar to the distribution of the gas molecules in the atmosphere or to the distribution of colloidal particles, as found by Perrin (19). In these cases equilibrium is established when the effect of gravity equals the osmotic pressure (or Brownian movement in the case of colloidal particles). For gases this is expressed by the formula

$$h = \frac{R}{g} \frac{T}{M} \ln \frac{P_0}{P} \quad (4)$$

where h is the height, P_0 the pressure at the bottom, P the pressure at height h , g the acceleration due to gravity, and M the molecular weight. In the case of ions dissociated by the colloidal micelle, equilibrium will be established when the electrostatic attraction of the inner layer equals the osmotic pressure of the dissociated ions. If this attraction were substituted for the force of gravity in the preceding equation, a similar expression would be obtained, according to which the concentration of the ions in the outer layer should decrease in geometric progression as the distance from the inner layer increases in arithmetic progression. Evidence in support of this theory will be presented later in this paper but the experiments of Bouyoucos and McCool (5), who found that as the water content of the soil decreases in an arithmetic progression the freezing point depression increases in a geometric progression, may be alluded to at this point.

From the migration velocities of the particles saturated with different bases, it is evident that the dissociation of the cations depends not only upon the valence but also upon other factors, among which atomic volume and the degree of hydration of the ions appear to be the most important, as has been shown by Wiegner (23). The smaller the volume of the cation the greater the hydration, and the greater the number of water molecules attached to the ion the smaller its potential and the more insensible therefore to the electrostatic pull from the surface. The higher the valence and the lower the hydration, on the other hand, the more easily the ion is attracted to the inner layer of opposite sign of charge. For each ion there appears to be a limiting potential in the double layer above which the ion cannot exist in the dissociated condition. It is significant that this limiting potential never exceeds the potential of the single common ions themselves, which is estimated by von Hevesy (10) to range about 70 millivolts. The potential calculated for the fastest moving soil particles saturated with NaOH was found to be about 56 millivolts, which corresponds to a migration velocity of 4μ per second in a potential gradient of 1 volt per cm. (15). Particles saturated with bases of higher molecular weight and valence always move slower, and this is true in cases where no excess base is added and

in the absence of other electrolytes. This is stated to preclude any argument that the effect is due to a Donnan equilibrium.

That the Donnan distribution depends only on the valence is a mathematical truth which no one will dispute. But the primary cause of certain colloidal behaviors, namely, the formation of an atmosphere of ions around a colloidal particle, is entirely independent of the Donnan equilibrium. The density and thickness of this ionic atmosphere or layer must be governed by the specific nature of the ions composing it. The Donnan distribution, which is an effect rather than a cause, does not enter until we have an excess of electrolyte (which is of course always the case to a certain extent, because of hydrolysis and the OH and H ions of the water).

On the basis of the foregoing theory we can easily account for the enormous swelling, or to be more exact—the imbibition of water, of the colloidal material when just saturated with NaOH (neutral reaction). The cation of this base remains highly dissociated. This causes a high osmotic pressure in the immediate neighborhood of the particles. Water is imbibed, or, what amounts to the same thing, the ions move farther away from the surface. This continues until there is an equilibrium between the electrostatic attraction and the osmotic pressure. The gel has attained a maximum in swelling. The same line of reasoning will explain why the electrokinetic potential is at a maximum when the material is just saturated with base. The law relating the charge e to the potential ζ of concentrically charged spheres of radius r and r_1 is

$$\zeta = \frac{e (r_1 - r)}{D r r_1} \quad (B)$$

For the potential of the double layer of colloidal particles, this is written

$$\zeta = \frac{e \delta}{D r (r + \delta)} \quad (C)$$

where D is the dielectric constant of the medium and δ is the "thickness" of the double layer, or the mean distance between the two layers.

THE HOFMEISTER SERIES

From this expression it is evident that the greater the number of charges, that is, the greater the number of ions of one sign of charge in excess of the number of ions of opposite charge, in the two layers the greater is the potential difference between them. Also the further away from the inner layer the ions of the outer layer are able to diffuse before equilibrium is established between the electrostatic and the osmotic forces, the greater also is the ζ . The different ability of the ions to diffuse into the outside medium against the electrostatic attraction depends upon the specific nature of the ions, such as hydration and valence. It

is undoubtedly in this relationship that the ions arrange themselves in the well-known Hofmeister or lyotropic ion series.

Loeb denies emphatically the existence of such ion series as far as the proteins are concerned, and others are attacking the work of Loeb because of this denial. It seems to the writer that there need be no conflict between the laws governing the Donnan equilibrium and the Hofmeister ion series. Both are independent of each other and both may be coexistent. It seems that the cart has been put before the horse. The Donnan distribution of the free electrolyte is an effect rather than a cause in colloidal behavior. The Donnan equilibrium is governed by another equilibrium which precedes it, namely, the equilibrium between the colloidal micelle and its ions. In view of the great differences in dissociation, solubility, and hydration, in common compounds, it would seem absurd to assume that all ions would bear the same relation to the colloidal micelle. The equilibrium between micelle and its ions must be governed by the specific nature of both. That the migration velocity is greater in the Na-saturated than in the K-saturated particles and that the velocity of the Ca-saturated is greater than the velocity of the Ba-saturated particles must depend upon differences in the nature of the ions. The atmosphere of Na ions extends farther out than the atmosphere of the K ions. The potential and also the quantity of water held by osmotic forces (the swelling) are correspondingly greater in the case of the Na-saturated particles. The same thing applies to the differences between any other pair of base-saturated colloids.

As an outside electrolyte enters into the system, a Donnan distribution is established, as measured in the foregoing case by the negative adsorption, and this will vary in the same order as the potential and the swelling. The addition of an electrolyte suppresses the potential, the swelling, and the inequality (the value of $\frac{x}{y}$) of the Donnan distribution.

The laws governing the Donnan equilibrium will be very briefly discussed here, the uninitiated reader being referred to original sources (6, 12). But instead of making use of the conventional membrane which separates a non-diffusible ion from the rest of the system, the case of a colloidal micelle will be here considered.

As an example, consider a colloidal particle saturated with NaOH and suspended in pure water and assume the entire absence of free electrolytes (a condition which is of course never realized). Some of the Na ions will dissociate and diffuse into the water until the backward electrostatic pull just balances the osmotic tendency of the ions to distribute themselves equally in all parts of the system. An ion atmosphere has been formed around the particle within which the water is osmotically held, is more or less rigid, and must therefore move with the particle to which it belongs. The particle, its atmosphere of ions, and its imbibed water constitute the colloidal micelle. Within this micelle

the intensity of the electric fields, and therefore the potential gradient, will vary as we pass from one to the other of the following phases of the system.

| | | | | |
|-------------------------|---------------------------------------|---------------------------|-------------------------------------|-------------------|
| interior of particle | interfacial layers of molecules | inner layer of ions | outer layer or ion atmosphere | outside liquid |
|-------------------------|---------------------------------------|---------------------------|-------------------------------------|-------------------|

The potential difference in the double layer, which depends on the number of ions dissociated and on the thickness of the atmosphere, will, like the imbibition, be at a maximum. The P.D. (potential difference) between the interior of the atmosphere and the outside water will likewise be at a maximum.

THE DONNAN EQUILIBRIUM

Now let an electrolyte like NaCl be added to the suspension and let us consider the Donnan distribution of ions between the micellar solution (inside the ion atmosphere) and the inter-micellar or outside solution after equilibrium has been established. The principle of a constant ion product demands that

$$[\text{Na}^+][\text{Cl}^-]_{\text{outside}} = [\text{Na}^+][\text{Cl}^-]_{\text{inside}}$$

where the brackets signify concentration.

If this equation is to hold it is evident that fewer of the Na and Cl ions will have distributed themselves inside the Na-ion atmosphere than in the outside solution. This is best expressed by the general equation for the Donnan equilibrium for monovalent ions

$$x^2 = y(y + z) \quad (D)$$

where, in application to the above case, x is the chloride, cation, and anion concentration in the (outside) intermicellar solution; y is the concentration of the same ions inside the micellar ion atmosphere; and z is the concentration of the Na ions of the micellar atmosphere dissociated by the colloidal particle.

In this expression $x > y$, that is, the Na and Cl ions must be "negatively adsorbed" by the soil colloidal material, as was shown in table 1, and the negative adsorption must be greater the greater the value of z , that is, the greater the number of ions dissociated by the colloidal particle.

If the values of x and y can be determined, z may be calculated

$$z = \frac{(x + y)(x - y)}{y} \quad (E)$$

when all ions are monovalent.

Loeb has shown that the P.D. across a membrane separating a non-diffusible ion from an outside solution containing no such ions is reduced by the addition of an electrolyte. It can similarly be shown that the P.D. between the interior

of the micellar atmosphere and the outside liquid must be suppressed by the addition of an electrolyte. By inserting the values of $\frac{x}{y}$, as determined by the Donnan equilibrium, in the Nernst formula we get

$$\text{P.D.} = \frac{R}{a} \frac{T}{F} \ln \frac{x^a}{y^a} = \frac{R}{F} \frac{T}{a} \ln \frac{x}{y}$$

where a is the valence.

By substituting the common in place of the natural logarithms and writing the numerical value for $\frac{R}{F} \frac{T}{a}$ at room temperature we get

$$\text{P.D.} = 58 \log \frac{x}{y} \text{ millivolts} \quad (F)$$

Now since the value of $\frac{x}{y}$ depends upon the magnitude of z as well as on the concentration of the added electrolyte, the effect of the latter on the P.D. can best be illustrated by writing $\frac{x}{y}$ in terms of y and z . When all the ions in the Donnan equilibrium have the same valence then

$$x^2 = y(y + z)$$

and

$$x = \sqrt{y(y + z)}$$

Substituting this value for x in the term $\frac{x}{y}$, we get

$$\frac{\sqrt{y(y + z)}}{y} = \sqrt{\frac{y + z}{y}} = \sqrt{1 + \frac{z}{y}}$$

Formula (F) then becomes

$$\text{P.D.} = \frac{58}{2} \log \left(1 + \frac{z}{y} \right) \text{ millivolts} \quad (G)$$

From this formula it is evident that the greater the concentration of electrolyte added, that is, the greater the value of y , z remaining constant, the greater the suppression of the P.D.

THE VALENCE EFFECT

Let us now see how a difference in the valence of the ions must, according to theory, affect the distribution of the ions between the micellar and intermicellar

solutions and, by virtue of this distribution, to what degree a suppression of the above discussed P.D. is brought about.

The difference in valence effect will depend on which of the ions of the added electrolyte has the higher valence. If this be the ion which has the opposite sign of charge to that of the colloidal particle the effect will be greater, while if it be the ion with the same sign of charge the effect will be smaller than in the case where all ions involved are monovalent.

The first case may be illustrated by considering the effect of CaCl_2 on a Ca-saturated soil colloid. (The Ca-saturated material is considered in this case so as to have a common ion and thus avoid complications.)

Let x be the molar concentration of Cl ions in the outside solution, y the concentration of the Cl ions in the micellar solution; then $\frac{x}{2}$ and $\frac{y}{2}$ will be the corresponding concentrations of the Ca ions of the free CaCl_2 while the concentration of Ca ions dissociated by the colloidal particle in the micellar solution becomes $\frac{z}{2}$. Since CaCl_2 dissociates into two Cl ions and one Ca ion, the Donnan equilibrium becomes in this case

$$x^2 \frac{x}{2} = y^2 \frac{(y+z)}{2} \quad (H)$$

$$x^3 = y^2 (y+z)$$

$$x = \sqrt[3]{y^2 (y+z)}$$

Expressing $\frac{x}{y}$ in terms of y and z we get

$$\frac{x}{y} = \frac{\sqrt[3]{y^2 (y+z)}}{y} = \sqrt[3]{\frac{y+z}{y}} = \sqrt[3]{1 + \frac{z}{y}}$$

and formula (F) becomes in this case

$$\text{P.D.} = \frac{58}{3} \log \left(1 + \frac{z}{y} \right) \text{ millivolts} \quad (I)$$

By comparing formula (G) and (I) it will be seen that, at the same values of y and z , the P.D. between the micellar and the outside solution in the case of an electronegative colloid will be only $\frac{2}{3}$ as great when the cation of the added electrolyte is divalent and the anion is monovalent as when both ions are monovalent. This difference is merely due to the suppressing effect of the added electrolyte and has nothing to do with the actual difference in P.D. in the case of the Ca- and Na-saturated colloid. The P.D. in the case of the Na-saturated particles is much higher at the outset, that is, the value of z is greater because of a greater dissociation of Na ions into the micellar atmosphere, as already pointed out.

Let us now consider the second case, namely, the effect of an electrolyte with a divalent ion having the same sign of charge as that of the colloidal particle while the other ion is monovalent. The effect of Na_2SO_4 on the Na-saturated soil colloid may be considered as an example. If x be the molar concentration of Na ions in the outside solution, y the concentration of Na ions of the Na_2SO_4 in the micellar solution; then $\frac{x}{2}$ and $\frac{y}{2}$ will be the corresponding concentrations of the SO_4 ions while z represents the concentration of the Na ions dissociated by the particle. The equilibrium equation then becomes

$$x^2 \frac{x}{2} = \frac{y}{2} (y + z)^2 \quad (J)$$

$$x^3 = y (y + z)^2$$

$$x = \sqrt[3]{y (y + z)^2}$$

substituting $\sqrt[3]{y (y + z)^2}$ for x in $\frac{x}{y}$, we get

$$\frac{\sqrt[3]{y (y + z)^2}}{y} = \sqrt[3]{\frac{(y + z)^2}{y}}$$

and formula (F) becomes in this case

$$\text{P.D.} = \frac{58}{3} \log \frac{(y + z)^2}{y^2} \text{ millivolts} \quad (K)$$

with the same values for y and z , this formula gives a P.D. which bears the same ratio to the P.D. given by formula (G) as 1.33 to 1.00. Na_2SO_4 will therefore suppress the P.D. less than NaCl . The addition of an electrolyte with an anion having a still higher valence will lead to a still lesser suppression of the P.D. For $\text{Na}_3\text{Fe}(\text{CN})_6$ and $\text{Na}_4\text{Fe}(\text{CN})_6$ the above expression for $\frac{x}{y}$ becomes

$$\sqrt[4]{\frac{(y + z)^3}{y^3}} \text{ and } \sqrt[5]{\frac{(y + z)^4}{y^4}}$$

respectively.

This negative influence upon the suppression of the P.D. and, as we shall see later, upon other properties as well, which must be encountered whenever the valence of the ion of the same sign of charge as that of the colloid is higher than the valence of the other ion has apparently been overlooked by Loeb. He considers only the cases represented by formula (G) and (I) in which the term $\log \left(1 + \frac{z}{y}\right)$ occurs and denies repeatedly that the ion of the same sign of charge as the colloid exerts any influence whatsoever (12, p. 203, 246). He states "that

whenever a salt depresses any physical property of a protein (or a colloidal solution in general) this action is due to that ion of the salt which has the opposite sign of charge to that of the protein ion." Further (p. 204) that "the term, $\log \left(1 + \frac{z}{y} \right)$ derived from the (Donnan) equilibrium equation makes the P.D. a function of z and y , i.e., that ion which has the opposite sign of charge to that of the protein ion."

But it is evident that according to Nernst formula (F) the P.D. is a function of

$$\frac{(\text{Cl}^-) \text{ outside}}{(\text{Cl}^-) \text{ inside}} = \frac{(\text{Na}^+) \text{ inside}}{(\text{Na}^+) \text{ outside}} = \frac{z}{y} = \frac{y+z}{x}$$

in the case of NaCl in equilibrium with the Na-saturated colloid. The P.D. is therefore a function of the relative distribution of either ion and this distribution varies, as we shall see, with the valence of either ion. The term $\left(1 + \frac{z}{y} \right)$ represents a special case derived from the value of $\frac{z}{y}$ in the equilibrium equation in the case of certain valence combinations [formulas (D) and (H)]. In other valence combinations $\frac{z}{y}$ in terms of y and z assumes different forms, as has been shown.

Curiously enough the experimental work of Loeb on the proteins, excepting certain data on anomalous osmosis and a few other cases, appears to support his contentions. In his paper on electrical charge and anomalous osmosis, he suggests however that salts with trivalent cations and tetravalent anions form "loose compounds with isoelectric gelatin" (11, p. 486).

Formula; (F) shows the P.D. to be a function of $\frac{z}{y}$ independently of the valence, whereas formulas (G), (I), and (K) show how the P.D. varies with the valence at given concentrations of y and z . It becomes evident therefore that the value of $\frac{z}{y}$ must be different in each of the cases presented. In other words, the distribution of the different ions in the Donnan equilibrium must be more or less unequal depending upon the valence, that is, the observed negative adsorption of a non-reaching ion will vary with the valence according to a definite rule.

The theoretical relationship between valence and the Donnan distribution is shown in table 2.

The first column in the table shows the condition of the colloid, which is assumed to be saturated with and dissociating the same cation as that of the salt added, as shown in the second column. The third column shows the corresponding equilibrium formulas. The fourth column gives the formulas by which the P.D. is calculated, whereas the fifth column gives the P.D. ratios

as far as they are governed by the Donnan equilibrium alone. The sixth column gives the distribution ratios of the ions of the salt between the micellar and intermicellar solutions when $y = z$.

The distribution ratios bring out the relation that the higher the valence of the anion (the valence of the cation remaining the same) the greater the relative concentration of the added electrolyte outside the micellar solution as compared to the concentration inside this solution. An increase in the valence of the cation (the valence of the anion remaining the same) has the opposite effect. In the former case the negative adsorption is increased; in the latter case it is decreased. It will be shown later that this relationship actually exists in the case of soil colloids.

TABLE 2

Theoretical relationship between valence, P.D. and ion distribution as demanded by the Donnan equilibrium between the micellar and intermicellar solution in an electronegative soil colloid

| CONDITION OF COLLOID | GENERAL FORMULA OF SALT ADDED | EQUILIBRIUM FORMULA | P.D. IN MILLIVOLTS = | P.D. RATIOS | DISTRIBUTION RATIOS $\frac{x}{y}$ when $y = z$ | $\log \frac{x}{y}$ |
|---------------------------------|-----------------------------------|---------------------|--|-------------|--|--------------------|
| M ⁺⁺⁺ —saturated.... | M ⁺⁺⁺ A ₃ ' | $x^3 = y^3 (y + z)$ | $\frac{58}{4} \log \left(1 + \frac{z}{y} \right)$ | 0.500 | 1.190 | 0.075 |
| M ⁺⁺ —saturated..... | M ⁺⁺ A ₂ ' | $x^2 = y^2 (y + z)$ | $\frac{58}{3} \log \left(1 + \frac{z}{y} \right)$ | 0.666 | 1.259 | 0.100 |
| M ⁺ —saturated..... | M ⁺ A' | $x = y (y + z)$ | $\frac{58}{2} \log \left(1 + \frac{z}{y} \right)$ | 1.000 | 1.414 | 0.150 |
| M ⁺ —saturated..... | M ₂ ' A'' | $x^2 = y (y + z)^2$ | $\frac{58}{3} \log \frac{(y+z)^2}{y^2}$ | 1.333 | 1.587 | 0.200 |
| M ⁺ —saturated..... | M ₃ ' A''' | $x^3 = y (y + z)^3$ | $\frac{58}{4} \log \frac{(y+z)^3}{y^3}$ | 1.500 | 1.682 | 0.225 |
| M ⁺ —saturated..... | M ₄ ' A'''' | $x^4 = y (y + z)^4$ | $\frac{58}{5} \log \frac{(y+z)^4}{y^4}$ | 1.600 | 1.741 | 0.241 |

THE THEORY OF SWELLING AND THE INFLUENCE OF VALENCE

It was suggested in the foregoing that the enormous imbibition of water (swelling) exhibited by the NaOH-saturated soil colloids is due to a greater dissociation, or, what amounts to the same thing, to a weaker association with the colloidal complex of this cation. As a particle becomes saturated with NaOH the density and therefore the osmotic pressure of the ion atmosphere reaches a maximum. This explains the maximum swelling at that point. If an excess of base or any other electrolyte is added the swelling is suppressed. It will be shown that this suppression of the swelling is governed by the valence as well as by the concentration. The Donnan equilibrium will account for this influence. Loeb, who has established a definite relation-

ship between the concentration of the free electrolyte and the swelling of proteins, admits no other influences of valence than that of the ions having an opposite sign of charge to that of the colloid. It will be shown first, that, according to theory, the valence of both ions must influence the suppression of the osmotic pressure and swelling brought about by the addition of an electrolyte. Later, it will be shown that this double influence actually exists in the case of soil colloids.

Let us first consider the addition of NaCl to the Na-saturated colloid. The equilibrium equation then becomes

$$x^2 = y(y + z)$$

It is evident that $y < x$, that $(y + z) > x$, and also that the sum of the unequals is greater than the sum of the equals, thus—

$$2y + z > 2x$$

The excess concentration in the micellar solution above that of the outside solution is therefore

$$2y + z - 2x \tag{L}$$

Since $2x > 2y$ it follows that

$$2y + z - 2x < z$$

the addition of the salt has therefore decreased the difference in osmotic pressure between the micellar and intermicellar solutions. The greater the concentration of the salt solution added the smaller the difference in osmotic pressure between the two phases. Loeb makes this very obvious, by putting

$$x = \sqrt{y^2 + yz} \text{ or } 2x = \sqrt{4y^2 + 4yz}$$

and

$$2y + z = \sqrt{4y^2 + 4yz + z^2}$$

when the difference becomes

$$\sqrt{4y^2 + 4yz + z^2} - \sqrt{4y^2 + 4yz}$$

from which it is readily seen that the difference decreases as y increases with the increase in salt added.

If the excess of molar concentration of ions in the micellar solution above that of the outside solution is represented by e , then

$$2y + z = e + 2x$$

When y and x equal zero $z = e$; the osmotic pressure and the swelling are therefore at a maximum in the absence of free electrolytes. Let us now consider

the influence of a salt in which the ion of the opposite sign of charge to that of the colloid is bivalent. The addition of CaCl_2 to the Ca-saturated colloid will serve as an example. The equilibrium equation then becomes

$$x^2 \frac{x}{2} = y^2 \frac{(y+z)}{2} \quad (H)$$

Since x and y represent the molar concentrations of the Cl ion outside and inside the micellar solution respectively, $\frac{x}{2}$ and $\frac{y}{2} + \frac{z}{2}$ will be the corresponding Ca-ion concentrations. The excess ion concentration in the micellar solution above that of the outside solution is therefore

$$e = 3/2 y + \frac{z}{2} - 3/2 x. \quad (M)$$

As an example of the influence of a bivalent ion having the same sign of charge as that of the colloid, the addition of Na_2SO_4 to the Na-saturated colloid may be given. The equilibrium equation becomes then, as already shown

$$x^2 \frac{x}{2} = \frac{y}{2} (y+z)^2 \quad (J)$$

In this case the molar concentration of the monovalent Na ion is x and $y+z$, outside and inside the micellar solution, respectively while the corresponding concentration of the SO_4 ion is $\frac{x}{2}$ and $\frac{y}{2}$ and the excess ion concentration in the micellar solution becomes

$$e = 3/2 y + z - 3/2 x \quad (N)$$

Without going into further details it might be stated that the corresponding expression in the case of a trivalent anion becomes

$$e = 4/3 y + z - 4/3 x$$

and in the case of a tetravalent anion:

$$e = 5/4 y + z - 5/4 x$$

From the foregoing it is evident that the suppression of the osmotic pressure or the swelling of colloids by the addition of electrolytes is a function of the valence of both of the ions as well as of their concentration. It will be shown that this influence expresses itself in the case of soil colloids either in a difference in the value of e or in a difference in swelling. When the gel is free to swell, e remains constant, whereas if the swelling is inhibited e is increased. This can best be understood after the experimental data have been presented and will then be discussed.

THE DISTRIBUTION OF IONS IN A BENTONITE GEL

In the experiment given in table 1 the concentration of the solution outside the gel, that is, the value of x only, was determined. Since the volume of the micellar solution was unknown the value of y could not be ascertained. In the following experiments the concentration of the solutions inside and outside the gel at equilibrium has been determined. This was done in the following manner:

Graduated cylinders of 100 cc. capacity were cut off at the 60 cc. division. Twenty-five cc. of the solution to be studied were placed in each cylinder and portions of the dried granulated soil colloid or bentonite¹ were added until all of the solution was imbibed. About two days was generally allowed to complete the imbibition. A parchmented Whatman diffusion shell was then thrust into the gel and 15 cc. of the same solution was placed inside the shell. This quantity left the level inside the shell somewhat below the level of the gel, thus creating a slight hydrostatic pressure within the gel. The latter could therefore never become flooded but was compelled to retain its water against a pressure represented by the difference in level.

The amount of liquid imbibed is not very constant but varies with the size of the granules and with the quantity added at any one time. If the granules and the quantities added are very large, partial voids may be formed within the gel, or a mass will result at the bottom of the cylinder so compact as to prevent a maximum swelling. The best results were obtained in the case of the highly swelling bentonite and the alkali-saturated soil colloids by using the granules which passed a sieve having 12 meshes to the inch but were retained by a sieve having 50 meshes. In the case of the materials saturated with the divalent cations a more homogeneous gel is formed by using more finely granulated material. The bentonite, which is not always uniform in its natural condition, was made into a thick paste and thoroughly homogenized by mixing. It was then dried at 110°C. and granulated as described.

When the colloid is allowed to swell under the conditions described in the foregoing we may look upon the liquid within the gel as belonging entirely to the micellar liquid. If the micelles with their osmotically held liquid envelopes retain their spherical form, this would not be strictly true as the interstices would then not belong to the micelles, but when the swelling takes place under restraint in a confined space with a limited amount of liquid it is probable that the micellar ions diffuse into the interstitial spaces. This is still more probable in the cases where an excess of colloid above that just needed to imbibe the 25 cc. solution was used.

The cylinders, tightly stoppered, were left to stand three days until equilibrium was established. Ten cubic centimeters were then withdrawn from the

¹ The sample of bentonite here used was the same as that previously studied by Anderson and Mattson (2). For a description and analysis of the soil colloids see authors' reference (13).

solution in the diffusion shell. The shell was then removed from the gel and about 10 gm. of the gel was rapidly placed in a crucible, provided with a lid, and weighed. The gel was then dried over night at 110°C. and again weighed. The weight of the imbibed water and of the dry colloid was thus obtained. The latter was then moistened with a normal solution of $MgCl_2$ or $MgSO_4$, depending upon which of the anions would not interfere with the analysis. This was

TABLE 3
The Cl ion distribution in the NaCl-bentonite system

| | | | | | |
|--|---------|---------|---------|---------|---------|
| x | 0.00641 | 0.01214 | 0.02830 | 0.05440 | 0.10600 |
| y | 0.00455 | 0.00925 | 0.02370 | 0.04840 | 0.09690 |
| $x - y$ | 0.00186 | 0.00289 | 0.00460 | 0.00600 | 0.00910 |
| z | 0.00448 | 0.00668 | 0.01009 | 0.01274 | 0.01905 |
| $\frac{x}{y}$ | 1.409 | 1.312 | 1.194 | 1.124 | 1.095 |
| $\log \frac{x}{y}$ | 0.1488 | 0.1181 | 0.077 | 0.0507 | 0.0395 |
| P.D..... | 8.63 | 6.85 | 4.47 | 2.94 | 2.29 |
| $e = 2y + z - 2x$ | 0.00076 | 0.0009 | 0.00089 | 0.00074 | 0.00085 |
| water | | | | | |
| colloid..... | 9.51 | 9.45 | 9.41 | 9.57 | 8.77 |
| M. E. cations dissociated per gram.... | 0.0427 | 0.0630 | 0.0952 | 0.1212 | 0.1670 |
| Per cent dissociation..... | 5.35 | 7.88 | 11.88 | 15.17 | 20.90 |
| (P.D.) z | 0.0387 | 0.0458 | 0.0451 | 0.0374 | 0.0436 |

TABLE 4
The NO_3 ion distribution in the $NaNO_3$ -bentonite system

| | | | |
|--------------------------|---------|---------|---------|
| x | 0.00130 | 0.00610 | 0.01190 |
| y | 0.00082 | 0.00430 | 0.00910 |
| z | 0.00124 | 0.00435 | 0.00646 |
| $\frac{x}{y}$ | 1.585 | 1.418 | 1.308 |
| $\log \frac{x}{y}$ | 0.2001 | 0.1516 | 0.1165 |
| P.D..... | 11.60 | 8.79 | 6.76 |
| $e = 2y + z - 2x$ | 0.00028 | 0.00075 | 0.00086 |
| water | | | |
| colloid..... | 9.91 | 9.59 | 9.45 |
| (P.D.) z | 0.0144 | 0.0382 | 0.0436 |

done to prevent excessive swelling and dispersion, and to make washing possible. After that the mass was transferred to the filter and washed with a hot 0.01 *N* solution of the magnesium salt until the free ions in the gel were removed. The filtrate, which usually measured 250 cc., was evaporated and the anion of the solution imbibed by the gel was quantitatively determined. The concentration of this ion in the gel at equilibrium was calculated after the grams of water imbibed were converted into cc. by dividing by the density of water at the tem-

perature of the laboratory. The result was taken to represent the concentration (y) of the anion in the micellar solution. The concentration (x) of the outside solution at equilibrium was found by analysis of the 10 cc. removed from the shell. To this solution the same amount of magnesium salt was always added.

This method of determining the distribution of the ions of the free electrolyte between the gel and the outside solution is only applicable when one of the

TABLE 5
The SO_4 ion distribution in the NaSO_4 -bentonite system

| | | | |
|---|---------|---------|---------|
| x | 0.01465 | 0.04694 | 0.11090 |
| y | 0.01040 | 0.03932 | 0.09840 |
| $x - y$ | 0.00425 | 0.00762 | 0.01250 |
| z | 0.00699 | 0.01196 | 0.01930 |
| $\frac{x}{y}$ | 1.408 | 1.194 | 1.127 |
| $\log \frac{x}{y}$ | 0.1485 | 0.0770 | 0.0518 |
| P.D..... | 8.61 | 4.47 | 3.00 |
| $e = \frac{3}{2}y + z - \frac{3}{2}x$ | 0.00062 | 0.00053 | 0.00055 |
| water colloid..... | 9.64 | 9.54 | 8.87 |
| M. E. cations dissociated per gram..... | 0.0673 | 0.1140 | 0.1710 |
| Per cent dissociation..... | 8.42 | 14.30 | 21.40 |
| (P.D.) z | 0.0602 | 0.0535 | 0.0578 |

TABLE 6
The $\text{Fe}^{+++}(\text{CN})_6$ ion distribution in the $\text{Na}_4\text{Fe}(\text{CN})_6$ -bentonite system

| | | | | |
|------------------------|----------|---------|---------|---------|
| Original solution..... | 0.01036 | 0.02592 | 0.05256 | 0.10748 |
| x | 0.01210 | 0.03056 | 0.05952 | 0.11432 |
| y (calculated)..... | 0.00935 | 0.02250 | 0.04798 | 0.10184 |
| $\frac{x}{y}$ | 1.294(?) | 1.358 | 1.240 | 1.123 |
| P.D..... | 6.50(?) | 7.71 | 5.42 | 2.92 |
| water colloid..... | 9.92 | 9.72 | 9.62 | 8.77 |

ions, the anion in the case of electronegative soil colloids, does not react in any way with the colloidal material. The author has previously shown that the Cl and SO_4 ions are not adsorbed by soil colloids in neutral and alkaline solutions. The same is true of the NO_3 and apparently also of the $\text{Fe}^{+++}(\text{CN})_6$ ions. The PO_4 ion is, however, adsorbed under all conditions of pH. Phosphates could therefore not be used in these experiments because, the adsorption being reversible, some of the adsorbed ions together with the free ions would be removed from the gel by the washing, giving a value for y which might be even

larger than x . In other words, the negative adsorption of the free ions, which nevertheless would exist, would be obscured by the positive adsorption.

Tables 3, 4, 5, and 6 show the concentrations of the Cl , NO_3 , SO_4 , and $\text{Fe}^{+++}(\text{CN})_6$ ions inside (y) and outside (x), the bentonite gel at equilibrium at varying concentrations of the respective sodium salt. The sodium salts were chosen because the sample of bentonite used was almost entirely a Na-saturated product.

One of the objects of these experiments was to determine the influence of valence upon the distribution of the ions. It was therefore thought desirable to keep the concentration of the bentonite in the gel constant. It was found that 2.5 gm. bentonite would imbibe 25 cc. of either solution up to 0.1 N . This quantity was therefore added to each cylinder and after imbibition the gel was homogenized by stirring with a glass rod before the diffusion shell was introduced. The differences in the $\frac{\text{water}}{\text{bentonite}}$ ratio in the gels found after equilibrium are due to osmotic readjustments with the water in the shells.

In calculating the value of z and the P.D., complete dissociation was assumed. In the case of the monovalent NO_3 and Cl ions

$$z = \frac{(x + y)(x - y)}{y^2} \quad (E)$$

and

$$\text{P.D.} = \frac{58}{2} \log \left(1 + \frac{z}{y} \right). \quad (G)$$

In the case of the divalent SO_4 ion

$$z = \left(\sqrt{\frac{x^2}{y}} \right) - y \quad (O)$$

and

$$\text{P.D.} = \frac{58}{3} \log \frac{(y + z)^2}{y^2}. \quad (K)$$

The P.D. may be calculated in all cases most simply independent of the valence, thus

$$\text{P.D.} = 58 \log \frac{x}{y}$$

All concentrations are in equivalents per liter and the P.D. is in millivolts.

The excess ion concentration in the gel over that of the outside solution is, in the case of the monovalent anions,

$$e = 2y + z - 2x \quad (L)$$

and in the case of the SO_4 ion

$$e = 3/2 y + z - 3/2 x$$

The tables further show the ratio of water to colloid in the gel, the milliequivalents of cations dissociated per gram bentonite, the per cent dissociation and the product (P.D.) z . The milliequivalents of cations dissociated per gram bentonite were calculated by dividing z , the average concentration of the dissociated cations in the micellar solution, by the number of grams of bentonite in 1 cc. of the gel; and the per cent dissociation is obtained by dividing the number milliequivalents dissociated per gram by 0.8, the total exchangeable cations in 1 gm., and multiplying by 100.

The experiments reveal a number of relationships of fundamental importance. Let the Cl ion distribution first be considered. The distribution of the Na ions of the NaCl or any other cations that may be displaced by the Na ions is of course the same as that of the Cl ions. x and y represent the chloride concentration outside and inside the gel respectively. Although the relative difference between x and y decreases with an increase in concentration, as shown by the values of $\frac{x}{y}$, the absolute difference between x and y increases with the concentration, amounting to 0.0091 N at a total concentration of about 0.1 N . This increase would not take place if z remained constant as one would expect it to. If anything, z might be expected to decrease because of a suppression of the dissociation by an increase in the cation concentration. On the contrary, one is surprised to find that z , as calculated from the equilibrium equation, increases with the concentration. This might lead some to doubt the applicability of the equilibrium equation to the cases here considered—but let us look a little farther. It will be seen that the P.D. decreases with the concentration and in such a way that the product of the P.D. and z is practically a constant.

How are we to account for this doubtless very significant fact? It will be shown in a later publication (part II) that the concept of ion activity becomes very useful in dealing with this problem. For the present it will be more practical to confine ourselves to the use of the term "potential." If the colloid adsorbs the base by virtue of the OH ions becoming attached to the interfacial layer of molecules, then we cannot speak of the cations as a product of ionization. Their presence in the micellar solution is merely the result of an electrostatic attraction following the adsorption of the OH ions. This adsorption of one in excess of the other of a pair of ions leads to a potential difference. This is the cataphoretic potential. Since this potential has never been found to exceed that of the common ions, it seems reasonable to assume that the adsorption of one ion in excess of the other is only possible within certain limits. The dissociated cations can remain free only below a certain potential. As the association of the OH ions with the interfacial layer proceeds further, an equiv-

alent of cations must be attracted by, and become associated with the inner layer of opposite charge; otherwise the P.D. would be much greater than is actually observed (14). If however the P.D. is reduced by the addition of an electrolyte some of the associated cations will be released, that is, the value of z will increase. This view offers an explanation why z increases with the concentration and why the product (P.D.) z is a constant.²

It is interesting to note that the excess of ions inside the gel as expressed by the difference $2y + z - 2x$ is also very nearly constant. This means that the difference in osmotic pressure between the inside of the gel and the outside solution is very nearly the same at equilibrium. We shall return to a discussion of this phenomenon in connection with later experiments.

From purely theoretical considerations based upon the electrokinetic behavior of the colloids, the author (13, 14) arrived at the conclusion that only a fraction of the exchangeable bases could exist in the dissociated condition. This conclusion is verified by these experiments as shown in the tenth row of the table. The per cent dissociated is, however, larger than it should be if we are to accept the formula (C) generally used to relate the charge of colloidal particles to the cataphoretic potential of the double layer. But since the number of free charges, that is, the number of ions dissociated, increases with an increase in concentration it is evident that this formula does not apply in its present simple form. The cataphoretic potential cannot be proportional to the number of ions dissociated but rather to their number multiplied by the activity coefficient (*see* part II).

In figure 1 the value of x , y , z , and e of table 3 are plotted as functions of x . If the apparently constant value of e , which equals about 0.0008 N holds for the lowest concentrations of x and y it is evident that the lower limit of $z = e = 0.0008$ when x and $y = 0$.

Turning now our attention to the distribution of the NO_3 ion we note that the figures in the last two columns of table 4 agree very closely with the corresponding figures in columns 2 and 3 in table 3. This agreement is in accordance with the Donnan equation since both ions are monovalent. The NO_3 ion was determined colorimetrically. For this reason the experiment covers only dilute solutions. In the case of the lowest concentration used (about 0.001 N) an abnormal drop in the value of z , e , and (P.D.) z will be observed. The explanation is the following. The bentonite contains a small but, in comparison to low concentrations, an appreciable quantity of sulfate. This will not affect the calculation of the P.D., which depends solely on the relative concentration of x and y , but the calculated value of z will be too low unless x and y represent absolute concentrations.

The economic significance of the negative adsorption is readily recognized in the case of the NO_3 ion. Everyone knows that nitrates are easily leached out

² A close examination of the data presented by Loeb (12, p. 186) discloses a similar behavior in the case of gelatin. This will be discussed in part II.

of the soil but the true cause of this loss has not been understood. We now see that the NO_3 ion is not only "weakly adsorbed" but negatively adsorbed inasmuch as the micellar solution constantly throws an excess of these ions into the outside solution, that is, the solution which moves by gravity and is easily lost.

This, by the way, leads us to recognize two forms of soil solution, the distinction between which is of fundamental importance. The micellar solution is an integral part of the micelle. Any attempt to remove this solution by

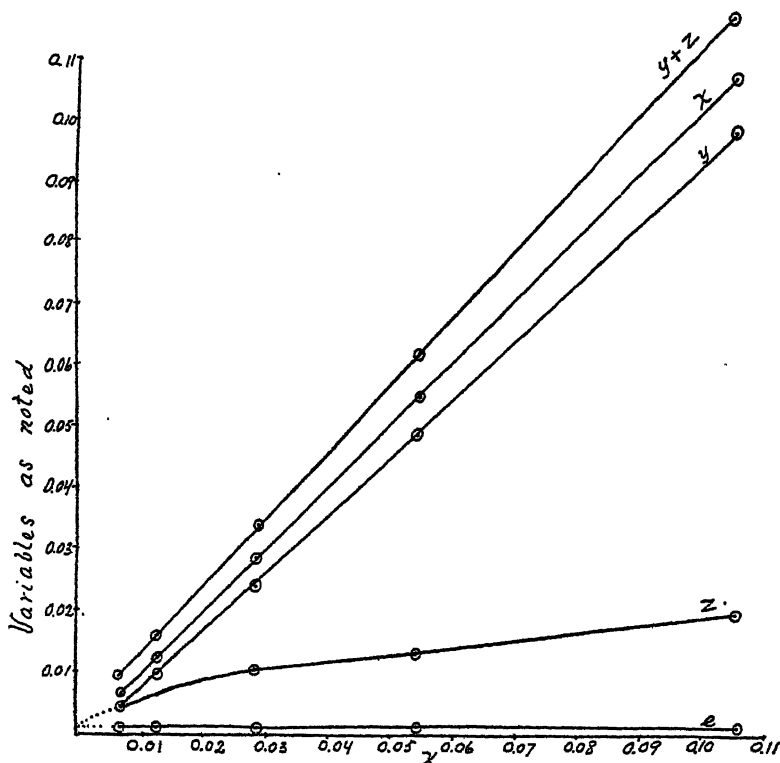


FIG. 1. THE VARIATIONS IN THE VALUES OF x , y , z AND e IN THE DONNAN DISTRIBUTION OF IONS IN A BENTONITE GEL AND IN THE OUTSIDE SOLUTION

pressure or any other means must fail. The micelle is like a living organism. It reacts to any change in the surrounding medium only more quickly because it has no membrane. Remove the water and the micelle is destroyed, leaving an inert particle comparable to the spore of an organism. This process is reversible. In dilute solutions (hypotonic) it swells; in strong solutions (hypertonic) it shrinks. It strives to maintain a definite osmotic pressure within its solution, as will be shown later. This analogy will be more evident as we proceed with our work.

As we study table 5 we observe differences in the various variables, as com-

pared to table 3 and 4, which are the direct result of the higher valence of the SO_4 anion. The value of z remains about the same at the corresponding concentrations. This is as it ought to be, considering that the $\frac{\text{water}}{\text{colloid}}$ ratio is also

nearly the same. The values of $x - y$, $\frac{x}{y}$, and the P.D. as compared with those of the corresponding concentrations in table 3 are very nearly as much higher as the theory demands. (Compare this with the theoretical differences in the case of mono- and divalent anions as given in the third and fourth row in table 2). The value of $e = 3/2 y + z - 3/2 x$ is again very nearly constant but is lower by about one third than this value in the case of the monovalent ions. The product (P.D.) z is of course higher since the P.D. is higher and z is the same, but here again the constancy appears to hold.

The experiment with the ferrocyanide was not very successful. When the gel was dried, this ion was partly decomposed, as evidenced by a blue coloration. The concentration within the gel (y) was therefore calculated from the concentration in the outside solution (x) and from that in the original solution. The volumes of the respective solutions were calculated from the volume of the original solution (40 cc.), the total weight of dry colloid (2.5 gm.), and the $\frac{\text{water}}{\text{colloid}}$ ratio. The ion was determined by the oxidation method with KMnO_4 .

According to theory the P.D. values at the same concentrations of the chloride and ferrocyanide should be in the proportion of 1.0 to 1.6 (compare table 2). This relationship is only approximated by the P.D. values in the third and fourth columns in table 6. The P.D. in the second column is even lower than in the case of the Cl ion, whereas the P.D. in the fifth column is somewhat lower than in the case of the SO_4 ion at the corresponding concentrations. It is safe to assume that these discrepancies are due to errors and that the relatively higher values in columns 3 and 4 represent more nearly the actual P.D.

The suppression of the P.D. ($= 58 \log \frac{x}{y}$) by the chloride, sulfate, and ferrocyanide of sodium is graphically represented in figure 2.

THE RELATION BETWEEN VALENCE AND SWELLING

In the last chapter we saw that the valence effect of the anions on the distribution of the electrolyte between the gel of an electronegative colloid and the outside solution expresses itself in such a way that the higher the valence the greater the difference between x and y . It will be recalled that in the above experiments the concentration of the colloid in the gel was about the same in all cases, the gels being prepared by allowing 2.5 gm. dry colloid to imbibe 25 cc. of the different solutions.

In continuing these experiments by employing still stronger solutions it was found that 2.5 gm. bentonite were no longer sufficient to imbibe the 25 cc. of the solutions and that the power of imbibition decreased more rapidly in the

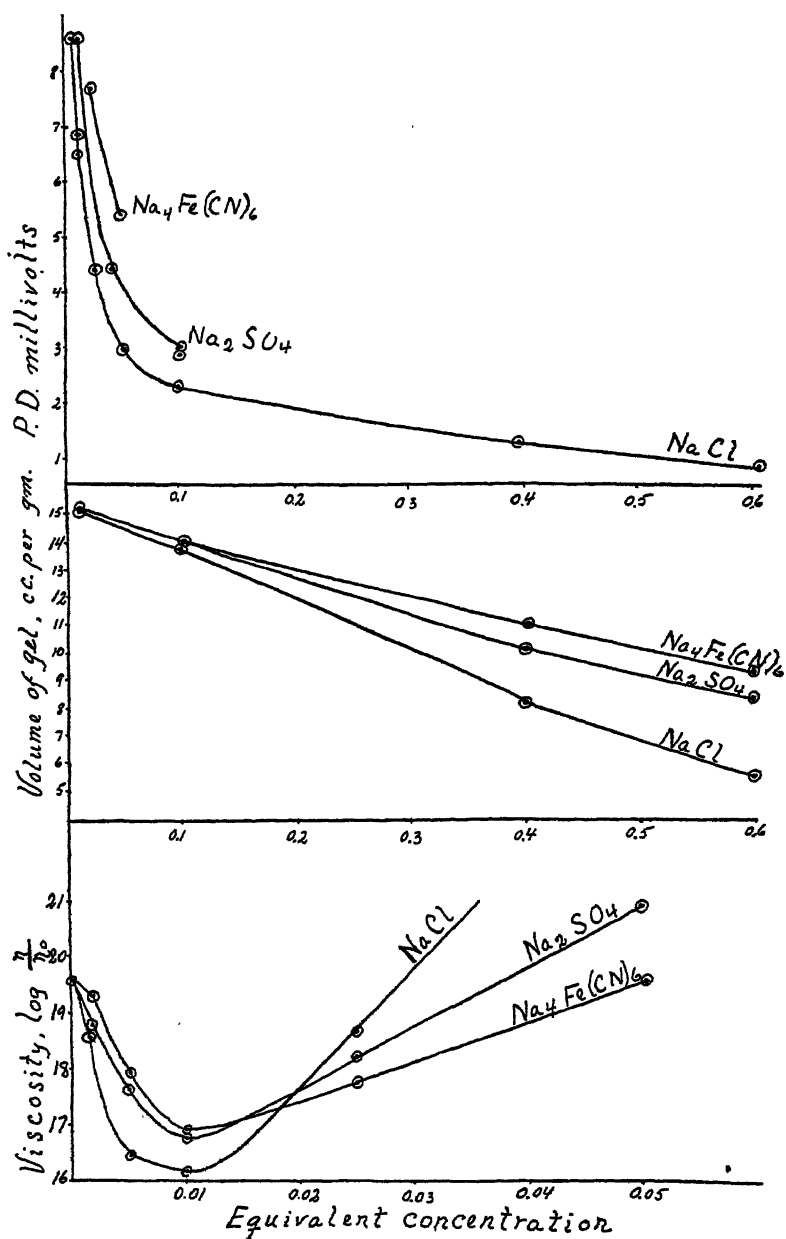


FIG. 2. THE INFLUENCE OF THE VALENCE OF THE ANION ON THE SUPPRESSION OF THE P.D. THE SWELLING, AND THE VISCOSITY OF BENTONITE

chloride than in the sulfate solution and in the sulfate solution more rapidly than in the ferrocyanide solution. These differences became very marked at concentrations about 0.4 to 0.6 *N*. The experiments were therefore conducted in such a way that additional small quantities of bentonite were gradually added to each cylinder until all of the 25 cc. was imbibed. The method is not very accurate but the resulting gels represented roughly a saturated condition. The diffusion shells were then inserted and filled with 15 cc. of the respective solutions. The distribution at equilibrium is given in table 7.

The most significant fact revealed by these experiments is that here the valence effect expresses itself, not in a difference in the values of $\frac{x}{y}$ and in a difference in P.D., as is the case when the concentration of the gel is kept constant, but chiefly in a difference in swelling as shown by the $\frac{\text{water}}{\text{colloid}}$ ratio.

TABLE 7

Distribution of the Cl, SO₄, and Fe⁺⁺⁺ (CN)₆ ions and the relation between valence and swelling

| | NaCl | | Na ₂ SO ₄ | | Na ₄ Fe(CN) ₆ |
|---|--------|--------|---------------------------------|--------|-------------------------------------|
| <i>x</i> | 0.4160 | 0.6256 | 0.4090 | 0.6110 | 0.4120 |
| <i>y</i> | 0.3960 | 0.6050 | 0.3894 | 0.5864 | 0.3920 |
| <i>x - y</i> | 0.0200 | 0.0206 | 0.0196 | 0.0246 | 0.0200 |
| <i>z</i> | 0.0410 | 0.0419 | 0.0298 | 0.0373 | |
| $\frac{x}{y}$ | 1.050 | 1.034 | 1.050 | 1.042 | 1.051 |
| P.D..... | 1.25 | 0.84 | 1.23 | 1.03 | 1.25 |
| <i>ε</i> | 0.0010 | 0.0007 | 0.0004 | 0.0004 | |
| $\frac{\text{water}}{\text{colloid}}$ | 4.72 | 3.06 | 7.25 | 6.18 | 7.68 |
| (P.D.) <i>z</i> | 0.0512 | 0.0352 | 0.0366 | 0.0384 | |

This is especially true in the case of the 0.4 *N* solutions. Here the values *x - y*, $\frac{x}{y}$, and the P.D. are almost identical, while the $\frac{\text{water}}{\text{colloid}}$ ratio bears the relation of 1.00, 1.33, and 1.42 in the case of the chloride, sulfate, and ferrocyanide respectively.

Since imbibition dilutes the micellar solution it is obvious that *z* must decrease as the swelling increases. The value of *z* is accordingly lower in the sulfate than in the chloride gel, the difference being such as to yield a more nearly uniform distribution ratio and P.D.

It appears that the swelling is markedly suppressed only at a concentration above 0.1 *N* whereas the P.D. is greatly affected in much lower concentrations. It seems, therefore, that the electrostatic stress is the one first to be undone by the effect of the electrolyte. After the P.D. has been considerably reduced the suppressing effect is transferred to the swelling. The latter, i.e.,

the imbibition, adjusts itself apparently in such a way as to keep the osmotic pressure, i.e., the value of e , constant.

The results of the above work may be summarized in the following statements:

When the concentration of the colloid in the gel is constant, that is, when the swelling is inhibited, the valence effect expresses itself in differences in the P.D.

When the gel is permitted to swell to a maximum the valence effect expresses itself in differences in the degree of swelling, i.e. in the quantities of water imbibed. It might be said that the gels tend to swell to the same P.D. in the same concentrations of the various salts.

TABLE 8
Effect of an interchange in colloid concentration on the distribution of Cl and SO₄ ions

| | NaCl | | | | Na ₂ SO ₄ | | | |
|-------------------------------|--------|--------|--------|--------|---------------------------------|--------|--------|--------|
| | A | | B | | A | | B | |
| Bentonite grams in 25 cc..... | 5.7 | 5.7 | 3.1 | 3.1 | 3.1 | 3.1 | 5.7 | 5.7 |
| x | 0.5222 | 0.5217 | 0.5132 | 0.5138 | 0.5136 | 0.5138 | 0.5310 | 0.5306 |
| y | 0.4941 | 0.4935 | 0.4953 | 0.4982 | 0.4849 | 0.4845 | 0.4745 | 0.4711 |
| $x - y$ | 0.0281 | 0.0282 | 0.0179 | 0.0156 | 0.0287 | 0.0293 | 0.0565 | 0.0595 |
| z | 0.0580 | | 0.0340 | | 0.0441 | | 0.0895 | |
| $\frac{z}{y}$ | 1.057 | | 1.034 | | 1.059 | | 1.122 | |
| P.D..... | 1.40 | | 0.83 | | 1.45 | | 2.91 | |
| e | 0.0016 | | 0.0006 | | 0.0006 | | 0.0025 | |
| water colloid | 3.27 | | 5.98 | | 6.06 | | 3.60 | |

When a system is at equilibrium and no work is done on the system, certain functions and the total free energy must be at a minimum. If the osmotic pressure alone governed the swelling the gel would swell indefinitely until $e = 0$. But since the osmotic pressure is opposed by an electrostatic attraction on the dissociated ions an equilibrium is established at a point where the osmotic pressure has been reduced to between 0.01 or 0.02 atmospheres.

(The osmotic pressure = $22.4 \times \frac{273 + t^\circ}{273} \times e$ atmospheres.) If the gel is

compressed or if it is prevented from swelling, both the P.D. and e will increase because of an increase in the value of z . The value of e will not increase, however, in proportion to the compression, because of the compensating effect of the Donnan distribution. Following the law of Le Chatelier and Braun the equilibrium of the system will be displaced in that direction which tends to undo the stress brought to bear upon it. When the gel is compressed the osmotic stress is kept down by forcing ions into the outside solution. But

this creates a higher P.D. and therefore an opposing stress. When the gel is free to swell, both the osmotic and the potential stresses attain a minimum through the imbibition of water.

This is shown more clearly by the following duplicated experiments. It was found by gradually adding bentonite to approximately 0.5 *N* NaCl and Na₂SO₄ solutions that 5.7 gm. of the dry substance was required to imbibe 25 cc. of the chloride, whereas 3.1 gm. sufficed to imbibe 25 cc. of the sulfate solution. In a duplicate series of cylinders the quantity of bentonite required to imbibe the chloride solution (5.7 gm.) was added to the sulfate solution, whereas the quantity required to imbibe 25 cc. of the sulfate solution (3.1 gm.) was added to the chloride solution. In the second series, the sulfate gel represents, therefore, a compressed gel whereas the chloride gel contained more liquid than would normally be imbibed. The gels were homogenized with a glass rod before the diffusion shells containing the respective solutions were introduced. The equilibrium distribution is shown in table 8.

In comparing the two A columns, which represent the conditions of equilibrium after free imbibition, we observe in a general way the same relationship

TABLE 9
Distribution of the Cl and SO₄ ions at the same $\frac{\text{water}}{\text{colloid}}$ ratio

| | x | y | $x - y$ | z | P.D. | e | $\frac{\text{WATER}}{\text{COLLOID}}$ |
|---------------------------------------|--------|--------|---------|--------|------|--------|---------------------------------------|
| NaCl..... | 0.4091 | 0.3870 | 0.0221 | 0.0455 | 1.40 | 0.0013 | 7.41 |
| Na ₂ SO ₄ | 0.4210 | 0.3895 | 0.0315 | 0.0481 | 1.96 | 0.0009 | 7.15 |

as in table 7. The swelling is very much greater in the case of the sulfate-treated gel. The greater swelling of this gel has reduced the value of z to such an extent that the difference between x and y , which directly depends upon z , is very nearly the same as in the chloride gel. The swelling has reduced the P.D. to the same value in both gels.

The figures in the B columns, representing dilution of the gel on the one hand and compression on the other, are very different. The results in the chloride (B) column are really not significant. All the figures are lower than in the chloride (A) column. This is to be ascribed to the presence of "outside" solution within the gel, y representing therefore not merely the micellar concentration but a mixture of x and y . The figures in the sulfate (B) column show a pronounced increase as the $\frac{\text{water}}{\text{colloid}}$ ratio was decreased from 6.06 to 3.60. The values of $x - y$, z , and the P.D. are about twice as high as in the A column, whereas e is four times greater.

In order to show the effect of the valence of the anions in higher concentrations when the ratio $\frac{\text{water}}{\text{colloid}}$ is constant, the experiment was modified as follows:

Two and a half grams of bentonite were allowed to imbibe 25 cc. distilled water, and the diffusion shells were put in place and filled with 15 cc. normal chloride and sulfate solutions. Because of the fact that all of the electrolyte was on one side of the membrane at the outset, 10 days were allowed for equilibrium. The results are shown in table 9.

These experiments leave no doubt that the valence of the ion of the same sign of charge as that of the colloid is a factor influencing the distribution of the ions and the suppression of the swelling. In the last experiment where the $\frac{\text{water}}{\text{colloid}}$ ratio differed only slightly, the difference between x and y (and the calculated P.D.) in the case of the two salts correspond fairly well with the difference demanded by the equilibrium equation, considering the analytical difficulties. It should be stated that the assumption of complete dissociation does not seriously affect the relative values in the case of the different salts at the same concentrations. The salts are ionized to about the same extent and the undissociated molecules distribute themselves equally throughout the

TABLE 10
Distribution of Cl and SO₄ ions within the same gel

| | x | y | $x - y$ | $\frac{x}{y}$ | P.D. | $\frac{\text{WATER}}{\text{COLLOID}}$ |
|--|--------|--------|---------|---------------|------|---------------------------------------|
| NaCl (A)..... | 0.2677 | 0.2464 | 0.0113 | 1.0457 | | 5.74 |
| NaCl (B)..... | 0.2580 | 0.2468 | 0.0112 | 1.0454 | 1.12 | 5.80 |
| Na ₂ SO ₄ (A)..... | 0.2606 | 0.2489 | 0.0117 | 1.0470 | | 5.74 |
| Na ₂ SO ₄ (B)..... | 0.2606 | 0.2494 | 0.0112 | 1.0450 | 1.13 | 5.80 |

system. The absolute difference between x and y as found by analysis is independent of the degree of ionization. The calculation of x is therefore unaffected, but the P.D., which depends upon the relative magnitudes of x and y , will in reality be higher than the calculated value.

Since the ions distribute themselves between the gel and the outside solution according to valence it seemed of interest to determine how this effect would express itself when ions of different valence are added to the same gel. For this purpose 0.5*N* NaCl and Na₂SO₄ solutions were mixed in equal proportions, making the solution 0.25 $\frac{N}{4}$ with respect to each salt. The experiment was carried out in duplicate, the results of which are shown in table 10.

The experiment in itself shows no apparent valence effect. The difference between x and y is the same within the limits of error, in the case of both ions. A comparison with table 8 (NaCl, B column, and Na₂SO₄, A column) will show however that the foregoing values of $\frac{x}{y}$ and of the $\frac{\text{water}}{\text{colloid}}$ ratio are all intermediate between the values obtained with NaCl and Na₂SO₄ separately

at the same concentration. In the last experiment there can only be one P.D. and one degree of imbibition for both electrolytes since both were present in the same system. The result represents therefore the algebraic sum of the action of both.

THE EFFECT OF THE HYDRATION OF IONS ON THE DONNAN DISTRIBUTION AND ON THE SWELLING

In a series of interesting experiments Wiegner (23) has shown that the addition of alcohol increases the power of the alkali cations to displace the divalent cations from the soil complex. Wiegner ascribes this effect to a dehydration of the otherwise heavily hydrated alkali cations. When freed from their water envelope the small alkali cations show a greatly increased displacing power. This increased displacing power simply means that the dehydrated ions are more strongly attracted by the inner layer of opposite sign of charge,

TABLE 11
The effect of alcohol on the distribution of the Cl ion in the LiCl-bentonite system

| Cc. 95 per cent alcohol in 100 cc..... | 20 | 40 | 60 | 80 |
|---|---------|---------|---------|------|
| x | 0.01548 | 0.01535 | 0.01490 | Lost |
| y | 0.01189 | 0.01230 | 0.01326 | |
| $x - y$ | 0.00359 | 0.00305 | 0.00164 | |
| z | 0.00826 | 0.00686 | 0.00348 | |
| $\frac{x}{y}$ | 1.300 | 1.248 | 1.124 | |
| P.D..... | 6.61 | 5.58 | 2.94 | |
| e | 0.00108 | 0.00076 | 0.00020 | |
| $\frac{\text{water}}{\text{colloid}}$ | 6.40 | 5.68 | 4.78 | 1.24 |
| Per cent dissociation..... | 6.62 | 4.87 | 2.07 | |

that the tendency of the dehydrated ions to remain dissociated in the micellar atmosphere has been lessened. Association at the interface takes the place of the association with the water molecules. The dehydrated ion is unstable, it cannot exist alone, its potential being too high.

This being the case, the effect of dehydration should show itself in the Donnan distribution, for if z is decreased because of a lessened dissociation the difference between x and y will be smaller. Also, the swelling should for the same reason decrease, apart from any direct dehydrating effect upon the colloid.

In the following experiment increasing quantities of alcohol were added to the solutions, LiCl being used instead of NaCl because of its greater solubility. (See table 11.)

The results of the experiment show the predictions to be fulfilled. The values of z , and therefore also $x - y$, $\frac{x}{y}$, and the P.D. decrease as the percentage of alcohol is increased, that is, as the cations are dehydrated. The percentage

of the exchangeable cations dissociated was calculated as described in the foregoing and shows a corresponding decrease. The quantity of water imbibed, i.e., the swelling, falls off very rapidly above 60 per cent alcohol. In this connection it may be added that 70 per cent alcohol flocculated a bentonite suspension completely without any electrolyte.

THE EFFECT OF COLLOID CONCENTRATION ON THE ION DISTRIBUTION IN THE Na_2SO_4 BENTONITE SYSTEM

In the following experiment the concentration of Na_2SO_4 was the same in all cases while the quantity of bentonite was increased from 2.5 to 3.5 gm. in each 25 cc. of the solution. The colloid concentration could not be carried beyond this point as the gel then became too stiff. (See table 12.)

Since a maximum swelling was prevented in all cases the effect of the variations in the concentration of the colloid shows itself in differences in the other

TABLE 12
The effect of colloid concentration

| Gm. bentonite in 25 cc..... | 2.5 | 3.0 | 3.5 |
|---|---------|---------|---------|
| α | 0.01448 | 0.01542 | 0.01559 |
| y | 0.01033 | 0.01041 | 0.00985 |
| $\alpha - y$ | 0.00415 | 0.00501 | 0.00574 |
| z | 0.00681 | 0.00836 | 0.01035 |
| P.D..... | 8.49 | 9.89 | 11.57 |
| e | 0.00058 | 0.00084 | 0.00174 |
| $\frac{\text{water}}{\text{colloid}}$ | 9.83 | 8.10 | 7.42 |
| $\frac{r_1}{r}$ | 2.990 | 2.814 | 2.737 |

variables. The values of z , $\alpha - y$, P.D., and e all increase with the decrease in the $\frac{\text{water}}{\text{colloid}}$ ratio. Since the sulfate concentration is constant, the value of z is the sole factor upon which all relationships hinge.

THE MICELLAR STRUCTURE

From the different values of z as calculated from the results of this experiment it will be possible to gain some information as to the distribution of the micellar cations in the micellar atmosphere. The magnitude of z as measured in the gel as a whole depends upon the number of micelles per unit volume and upon the average micellar ion concentration within the individual micelles. Since the three gels in the foregoing experiment all contained less water than would be freely imbibed osmotically, it is safe to assume that the micelles occupy a closely packed position and that all the liquid within the gels represents micellar liquid. Now since the quantity of liquid permitted to be imbibed was different in the case of each gel it follows that the thickness of the micellar

atmosphere differed accordingly, being thickest in the least concentrated gel and thinnest in the most concentrated gel. Although the size of the colloidal particles is unknown the relationship between the average radius r of the particles and the radius r_1 of the micelles may be calculated from the $\frac{\text{water}}{\text{colloid}}$

ratio, a specific gravity of 2.65 being assumed for bentonite. The $\frac{r_1}{r}$ ratios thus found for the three gels are given in table 12. Since $r_1 = r +$ thickness of the micellar atmosphere, this thickness is $1.99 r$, $1.814 r$, and $1.737 r$, respectively. The corresponding z values are 0.00681, 0.00836, and 0.01035. These figures represent, of course, the average concentrations within the micellar atmosphere of thickness $r_1 - r$ and not the concentration at any one point.

It is evident that the concentration or ion density in the micellar atmosphere decreases rapidly with the "height," i.e., the distance from the surface of the particle. Near the surface the concentration must be very high whereas in the outermost strata of the atmosphere the density approaches zero at the point of maximum imbibition. The same statement applies to the P.D. between the interior of the atmosphere and the outside solution. This P.D. must increase with the depth of the atmosphere, being greatest near the surface of the particle. The P.D. as calculated from the free ion distribution represents therefore, only the average P.D. for the gel as a whole. Within the micelle, y will decrease as z increases, hence the calculated P.D. will grow larger with the concentration of the gel. This explains why the P.D. between the gel and the outside solution does not exceed 12 millivolts even in the most concentrated gel while the cataphoretic potential may be several times greater. It seems probable that the limiting value for the foregoing P.D. (the Donnan potential) should bear a definite relationship to the cataphoretic potential but this will be discussed more profitably in connection with cataphoresis.

From the preceding $\frac{r_1}{r}$ ratios and from the corresponding z values, it would be possible to calculate the variation in ion density with the height in the micellar atmosphere, but because of the very narrow interval covered by the $\frac{r_1}{r}$ ratios, any error in the z values would be so magnified as to render such computations worthless. The foregoing experiments and calculations make it quite evident that the outer Helmholtz layer is not only diffuse, as first pointed out by Gouy (9), but that the ion density decreases in some inverse proportion to the distance from the particle.

THE BEHAVIOR OF ELECTRODIALYSED BENTONITE AT VARIOUS DEGREES OF SATURATION WITH NaOH

In the preceding experiments only the natural untreated bentonite was used. For the following experiment the bentonite was electrodialed until all the exchangeable bases were removed. The exchange capacity of the original

material was 0.803 milliequivalents per gram. Subsamples of the electro-dialyzed sample were saturated to the extent of 0.2, 0.4, 0.6, and 0.8 milliequivalents of NaOH per gram. Portions of the granulated materials were gradually added, each to 25 cc. of a Na_2SO_4 solution, until all of the liquid was imbibed. The experiment with the diffusion shells was then continued, as has been described. The series was extended, however, to include four cylinders to which an excess of NaOH was added. Since the major portion of it would remain unadsorbed, the excess base was added to the sulfate solutions to which bentonite, saturated to the extent of 0.8 milliequivalents per gram, was added, as before in quantities sufficient to imbibe the 25 cc. After equilibrium was established the sulfate concentration was determined inside (y) and outside (x) the gel. The results are given in table 13.

TABLE 13

The SO_4 ion distribution in electro-dialyzed bentonite saturated with various quantities of NaOH

| M.Eq. NaOH per gram... | 0.0 | 0.2 | 0.4 | 0.6 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
|---|---------|---------|---------|---------|---------|------------------|---------|---------|---------|
| Excess NaOH in Na_2SO_4 solution... | | | | | | 1.0 | 2.0 | 4.0 | 20.0 |
| x | 0.01435 | 0.01409 | 0.01336 | 0.01396 | 0.01439 | 0.01375 | 0.01413 | 0.01353 | 0.01401 |
| y | 0.01203 | 0.01091 | 0.01030 | 0.01063 | 0.01053 | 0.01096 | 0.01131 | 0.01143 | 0.01341 |
| $\frac{x}{y}$ | 1.193 | 1.290 | 1.300 | 1.313 | 1.366 | 1.255 | 1.249 | 1.184 | 1.045 |
| z | 0.00364 | 0.00509 | 0.00492 | 0.00537 | 0.00629 | Not determinable | | | |
| P.D..... | 4.44 | 6.41 | 6.61 | 6.86 | 7.85 | 5.72 | 5.60 | 4.26 | 1.11 |
| e | 0.00016 | 0.00032 | 0.00033 | 0.00037 | 0.00050 | | | | |
| water colloid..... | 2.99 | 4.07 | 7.94 | 8.74 | 8.90 | 8.38 | 6.98 | 6.48 | 2.76 |
| Per cent cations dissociated. | 1.35 | 2.59 | 4.88 | 5.88 | 7.00 | | | | |

The most significant fact revealed by this experiment is the existence of points of maxima with respect to the value of $\frac{x}{y}$ and the P.D. as well as to the quantity of water imbibed and that these maxima coincide with the quantity of NaOH corresponding to the base exchange capacity or 0.8 milliequivalents per gram. This relationship is not surprising and could even be predicted on the basis of the author's earlier work (15).

The results, graphically represented in figure 3, are to be interpreted as follows: In the electro-dialyzed material the cations in the micellar atmosphere are H ions except for some exchange with the Na ions of the added sulfate. The H ion, with its single molecule of water with which it is supposed to associate, is very small and possesses, accordingly, a high potential. It responds readily, therefore, to the electrostatic attraction of the inner layer. The result

is a low ion concentration in the micellar atmosphere, a low P.D., a low osmotic pressure, and a low imbibition. As the H ions are displaced by the Na ions there is an increase in the micellar ion concentration, the P.D. is increased, and the bentonite gradually regains its original power to swell, which attains a maximum at the neutral point. This explains the ascending part of the curves representing the P.D., the swelling and, as we shall see later, also the viscosity.

On the alkaline side of the neutral point the colloid continues to adsorb some of the base but the augmenting effect of this on the P.D., the osmotic pressure, and the swelling is more than offset by the suppressing effect of the free base. The excess, or free base, acts exactly as any other added electrolyte, hence the descending path of the curves.

The suppressing effect in this experiment was entirely due to the excess base and not to the added sulfate, which was kept constant except for fluctuations due to impurities. The sulfate was merely added as a convenient method of determining the equilibrium ratio $\frac{x}{y}$.

The determination of the concentration of any anion inside and outside the gel at equilibrium must give the same value for $\frac{x}{y}$. If the anion enters into combination with the colloid then the method here used of removing the free anions from the gel by washing is of course not applicable, since then some of the combined anions would also be removed. The OH ions could therefore only be determined in situ without disturbing the equilibrium, as with the hydrogen electrode. The equilibrium ratio would also be expressed by the hydrogen-ion concentrations inside and outside the gel. In the case of the electronegative colloid the cation concentration is greater inside the gel than in the outside solution, the concentration inside the gel being $y + z$, whereas in the outside solution it is x . The foregoing ratio would therefore be expressed by

$$\frac{(\text{H}^+) \text{ inside}}{(\text{H}^+) \text{ outside}} = \frac{y + z}{x}.$$

This equals $\frac{x}{y}$, as has been shown. Since

$$\text{pH inside} = -\log (y + z)$$

and

$$\text{pH outside} = -\log x$$

$$\log \frac{y + z}{x} = \text{pH outside minus pH inside.}$$

The use of the hydrogen electrode was however precluded because of the solidity of the gels. In systems of greater fluidity the method has been used with the result that a higher hydrogen-ion concentration was invariably found within the electronegative suspension than in the outside solution in equilibrium with the suspension. In the case of soil colloids high in sesquioxides which become electropositive in acid solutions the hydrogen-ion concentration is higher in the outside solution.

In the preceding experiment the value of x could be calculated from the sulfate-ion distribution only in the cases where the sulfate was the only free electrolyte present. The relative concentration of the free hydroxide inside and outside the gel must be the same as that of the sulfate, that is, the value of $\frac{x}{y}$ is the same for both. In calculating x the absolute concentrations must be known.

THE INFLUENCE OF THE NATURE OF THE EXCHANGEABLE CATION AND OF THE COMPOSITION OF THE COLLOID

In the previous experiments bentonite was exclusively used because it lends itself admirably in its natural condition to investigations of the nature here dealt with, is easily obtained, and saves the laborious preparation necessary in the case of soil colloids. In its colloidal behavior bentonite resembles closely the soil colloids having the same ratio of silica to sesquioxides.

In the following experiment it was thought desirable to employ soil colloids in order to meet the possible objection that what applies to bentonite may not apply to soil colloids and also to show the influence of a difference in the silica sesquioxide ratio. The Sharka and the Norfolk soil colloids, which have been extensively used in experiments previously reported, were accordingly selected.

The Li-, Na-, and K-saturated colloids were prepared by saturating the electrodialyzed materials with the respective hydroxides, whereas it was more convenient to prepare the Mg-, Ca-, and Ba-saturated materials by the neutral salt treatment.

The experiment was carried out by adding small portions of the granulated colloids to 25 cc. of the respective chloride solutions until all of the liquid was imbibed. The diffusion shells were then thrust into the gels and filled with 15 cc. of the chloride solution. The Cl-ion concentration in the gel and in the outside solution at equilibrium was determined gravimetrically. The results are shown in table 14.

In spite of a great deal of painstaking work the results of the experiment are not so conclusive as might be desired but, considering the difficulties encountered, a better relationship could hardly be anticipated by a repetition of the work. The results are nevertheless in a general way in agreement with the theory and even in the case of certain discrepancies, as in those of the K-saturated gel, there is something that should not be overlooked.

The alkali-saturated colloids, especially those which, like the Sharkey, contain a little humus, shrink upon drying into an extremely hard, compact mass. The humus, which does not readily return to the colloidal condition, seems to cement the particles together so that a comparatively strong osmotic force is required to overcome the cohesive force and thus again unlock the aggregates. This osmotic force was strong enough in the case of the more highly dissociated Li- and Na-saturated colloids, which readily imbibed the water, forming voluminous gels. The K-saturated gel showed, however, no tendency whatever to swell, as is shown by the $\frac{\text{water}}{\text{colloid}}$ ratio, which is about equal to that of the Na-saturated material. When not previously dried, the

TABLE 14
The influence of the exchangeable cation and of the composition of the soil colloids

| MATERIAL | SHARKEY | | | | | | NORFOLK: |
|-----------------------------------|---------|---------|---------|--|---------|---------------------------------------|----------|
| | Li | Na | K | Mg | Ca | Ba | Na |
| Exchangeable cation..... | | | | | | | |
| | 0.01513 | 0.01276 | 0.01206 | 0.01102 | 0.01034 | 0.01088 | 0.01164 |
| | 0.01192 | 0.01053 | 0.00786 | 0.00849 | 0.00877 | 0.00952 | 0.00875 |
| | 0.00728 | 0.00493 | 0.01064 | 0.01007 | 0.00779 | 0.00469 | 0.00673 |
| | 1.27 | 1.21 | 1.53 | .30 | 1.24 | 1.14 | 1.33 |
| P.D. | 6.02 | 4.81 | 10.72 | 1.61 | 5.33 | 3.36 | 7.18 |
| | 0.00086 | 0.00047 | 0.00224 | 0.00124 | 0.00079 | 0.00031 | 0.00095 |
| | 6.83 | 5.17 | 1.36 | 1.59 | 1.45 | 1.33 | 1.61 |
| Per cent dissociated per gram.... | 0.0499 | 0.0256 | 0.0145 | 0.0160 | 0.0113 | 0.0062 | 0.0108 |
| Per cent dissociation..... | 6.25 | 3.20 | 1.81 | 2.00 | 1.41 | 0.78 | 5.15 |
| | | | | $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ | | EXCHANGE CAPACITY MILLIEQUIV./GRAM | |
| Sharkey..... | | | | 3.18 | | 0.796 | |
| Norfolk..... | | | | 1.63 | | 0.207 | |

K-saturated gel swells considerably. Bentonite saturated with potassium swells considerably even after being dried (15). This material contains no protective colloid like humus and does not become so compact when dried as do the soil colloids. The aggregates are therefore more easily unlocked by the osmotic forces. It is interesting to note that the failure of the K-gel to swell shows itself in a high micellar ion concentration (μ), in a high P.D., and in a high osmotic pressure (π) as compared to the gels saturated with the other monovalent cations. The per cent dissociation, however, is lowest in the K-gel, which is in agreement with the hydration theory of Wiegner and with the energy of displacement as found by Gedroitz. The order of hydration and dissociation is: $\text{Li} > \text{Na} > \text{K}$, whereas the order of the flocculating and displacing power is the reverse.

When comparing the action of the divalent cations with that of the monovalent, reference should be made to formulas (E), (G), and (H) and to the second and third row in table 2. In the case of the divalent cations

$$z = \frac{x^3}{y^2} - y$$

It will be noted that the values of z , the P.D., and e fall off rapidly with an increase in the atomic weight of the divalent metals. This is not in harmony with the foregoing observation that the gels strive to adjust themselves, within certain limits, to the same potential by swelling or shrinking when free to do so. If the Bagel were more concentrated all the preceding values would be greater. Since the $\frac{\text{water}}{\text{colloid}}$ ratios do not differ very much in the case of the three gels we may conclude that we are here approaching a limiting value in gel concentration. It is evident that if the osmotically imbibed water is less than the volume of the capillaries, the volume of the gel is no longer affected by the osmotic forces. In a crude way the case is analogous to van der Vaal's correction of the gas formula. When dealing with the phenomena of swelling we must take into account the irreducible volume occupied by the dispersed phase and of the interstices. The apparent swelling, especially in the case of the Ba gel must therefore be appreciably greater than the true volume of osmotic imbibition, a fact which considerably distorts the relationships.

The per cent divalent cations dissociated is again in the order of the atomic weight.



This is again the order of the lyotropic series and is the reverse of the order of the displacing power as found by Gedroiz. It is evident that the least dissociated cation must form the most stable complex.

The influence of the composition of the colloid, that is, the ratio of silica to sesquioxides, shows itself by comparing the equilibrium condition of the Norfolk to that of the Sharkey colloid. The former, with a composition ratio of 1.63, has an exchange capacity only about one-fourth that of the latter, in which the composition ratio is 3.18. The number of cations dissociated per gram of the Norfolk colloid is therefore less than in the case of the Sharkey. The result is that the Norfolk gel attains an equilibrium distribution with less imbibition, i.e. with less swelling than is required by the Sharkey gel. At the same concentrations of the Na saturated Norfolk and Sharkey gels, that is, under conditions of inhibited swelling, the negative adsorption, i.e. the value of $\frac{x}{y}$, would be considerably greater in the case of the Sharkey, whereas under conditions of free imbibition the effect of the greater ion content of the Sharkey shows itself chiefly in the form of a difference in swelling.

The valence effect, it will be recalled, expresses itself in the same way. At a constant gel concentration, the SO_4 ion gave rise to a greater $\frac{x}{y}$ ratio than the Cl ion, whereas under conditions of free imbibition the valence effect showed itself in a greater swelling of the sulfate-treated gel. The valence effect of the cations follows the same rule but is, as the theory demands, reversed, the suppressing effect of the divalent cations being greater than that of the monovalent. The suppressing effect of the cations, i.e. the ions in combination with the electronegative colloid, is twofold however. In the first place there is the effect of the degree of dissociation, which varies with each cation, giving rise to the well-known and unrefuted Hofmeister series, or the so-called lyotropic

TABLE 15
Effect of valence and concentration on the viscosity of a bentonite suspension
Relative viscosity (water = 1)

| | CONCENTRATION | | | | | | |
|---|---------------|-----------|---------|--------|---------|--------|-------|
| | 0.0 N | 0.00167 N | 0.005 N | 0.01 N | 0.025 N | 0.05 N | 0.1 N |
| NaCl..... | 1.57 | 1.53 | 1.46 | 1.45 | 1.54 | 1.85 | 2.36 |
| Na ₂ SO ₄ | 1.57 | 1.54 | 1.50 | 1.47 | 1.52 | 1.62 | 1.77 |
| Na ₄ Fe(CN) ₆ | 1.57 | 1.56 | 1.51 | 1.48 | 1.51 | 1.57 | 1.64 |

TABLE 16
The effect of NaOH on the viscosity of electro dialysed bentonite

| | | | | | | |
|------------------------------|------|------|------|----------|----------|----------|
| NaOH m.equiv. per gram..... | 0.0 | 0.2 | 0.4 | 0.6 | 0.7 | 0.8 |
| Relative viscosity..... | 1.08 | 1.14 | 1.28 | 1.29 | 1.29 | 1.24 |
| NaOH m. equiv. per gram..... | 0.9 | 1.0 | 1.2 | 1.6 | 2.0 | 2.4 |
| | | | | ∞ | ∞ | ∞ |
| Relative viscosity..... | 1.23 | 1.21 | 1.19 | 1.18 | 1.20 | 1.76 |

series of ions. In the second place there is the effect of the Donnan distribution which is purely a valence effect.

The preceding experiment with different cations was, as already explained, not very successful, but allowing for the inherent discrepancies discussed and for the unavoidable errors in removing so small quantities of electrolyte from large volumes of gel, the results agree in a general way with the theory.

VISCOSITY

The viscosity of bentonite suspensions was determined by the use of an instrument similar in construction to the Ostwald viscometer. The time of outflow of distilled water at room temperature was 23 seconds. In one experiment the time of outflow of 5.52 per cent suspensions of untreated bentonite was determined in the presence of varying concentrations of the chloride, sulfate, and ferrocyanide of sodium. In another experiment the time of

outflow of 5.36 per cent suspensions of electrodyalized bentonite to which varying quantities of NaOH had been added was determined. The suspensions were prepared from more concentrated stock suspensions as follows:

The quantity of the stock suspensions which would be delivered from a 25-cc. pipette within a fixed time allowed for drainage was separately determined. This quantity was then placed in a series of test tubes to which the calculated amount of electrolyte was now added, making a total volume of 30 cc. The tubes were then shaken and the measurements made. The results, as expressed in the terms of relative viscosity $\frac{\eta}{\eta_0}$ (water = 1), are given in tables 15 and 16.

The experiment with the three-salt solutions shows a suppressing effect up to a concentration of 0.01 *N*. At concentrations of 0.025 *N* and above the viscosity increases again rapidly. This phenomenon is easily explained. According to the theory of Einstein (7) and of Arrhenius (3), the viscosity is a function of the relative volume occupied by the solute (or colloid). It has been shown by Loeb (12) that the viscosity of protein solutions fits the theory in a general way.

If this relationship holds for soil colloids as well, it follows that the volume occupied by the bentonite is smaller in a 0.01 *N* solution than in less concentrated solutions. This assumption is quite justified on the basis of the preceding data on swelling. The swelling of the gel must be a direct expression of the volume of the individual micelles. The suppression of the swelling with an increase in concentration is an effect of a shrinkage in the volume of the micelles due to a decrease in the osmotic pressure, brought about by the Donnan equilibrium.

This theory is further supported by the effect of the different salts. The viscosity suffers the greatest suppression in the presence of the chloride, whereas the suppression is least in the presence of the ferrocyanide, the effect of the sulfate being intermediate. This is the same valence effect encountered in the swelling of the gels and in the Donnan equilibrium.

It remains to explain the increase in viscosity at and above a concentration of 0.025 *N*. At very high concentrations (not shown in table 15) the suspensions set into a gel. That this increase in viscosity is the direct result of aggregation is evident from the fact that the concentrations at which the increase commences coincide with the concentrations sufficient to flocculate the suspension. It will be noted that the chloride causes a more rapid increase in the viscosity than the sulfate and the sulfate a more rapid increase than the ferrocyanide, the differences being more marked in the higher concentrations. It will be shown (part II) that this represents the order in which the three electrolytes cause the suspension to flocculate. The increase in the viscosity in higher concentrations must therefore be ascribed to the impeding effect of the micellar aggregates upon the flow of the liquid. Were it not for this aggregation the viscosity would continue to decrease with an increase in the

concentration just as the volume of the individual micelles decreases, as shown by the swelling.

Directing our attention to table 16 we meet with another turning point in that we find two minima and to maxima in the viscosity. We will at once perceive a parallelism between the viscosity in this experiment and the ion distribution and the swelling in the experiment with electrodyalized bentonite as given in table 13. In the latter experiment the second maxima is, however, absent.

Keeping in mind the theory of the soil colloid micelle propounded in the foregoing, the phenomena are easily accounted for. The electrodyalized micelle contains only H ions in the micellar atmosphere. The concentration of this ion is smaller than that of the Na ion. The equilibrium between the opposing forces, i.e. the osmotic and the electrostatic is attained with less imbibition than in the case of the Na-saturated micelles. The micelles occupy therefore a relatively smaller volume in the electrodyalized condition, resulting in a low viscosity. As the material is progressively saturated with NaOH the micellar ion concentration is increased, more liquid is imbibed and the volume of micelles becomes greater, which expresses itself in the form of a greater viscosity. The viscosity reaches a maximum at the proportion of 0.6 milliequivalents NaOH per gram colloid, which is somewhat less than the base exchange capacity.

With an excess of NaOH there is a drop in the viscosity as in the preceding experiments with the different salts. This is of course due to the suppressing effect of the free electrolyte, as has been explained.

In the proportion of 1.6 milliequivalents NaOH, or more, per gram bentonite the suspensions set into a gel, the relative viscosity approaching infinity as indicated in the table. When shaken vigorously the gels became temporarily quite fluid and could be run through the viscometer. The results of these measurements are shown beneath the infinity signs. This phenomenon is evidently an expression of the time required in the process of aggregation.

PLASTICITY

As the colloid concentration of a suspension is increased the viscosity increases gradually until at a certain concentration, depending upon the composition of the colloid and the nature of the exchangeable cation, a sudden great increase in viscosity is observed. The viscous flow as measured by the viscometer becomes all at once infinitely slow. This break in the viscosity curve marks the transition from a viscous to a plastic condition. The abruptness of this transition may be readily visualized. As long as the micelles can freely move about in the suspension medium, fluidity is maintained and the suspension obeys the law of liquids. When the micelles become so numerous that they occupy all the space and are in actual contact, free fluidity is no longer permitted. They are no longer in suspension, but rest, so to speak, on top of one another. We are entering the range of the formative or plastic

condition. At first the mass is very soft and offers but slight resistance to a change in form, but as the $\frac{\text{water}}{\text{colloid}}$ ratio is still further reduced the mass becomes more stiff and formative. This phenomenon is in harmony with the previously

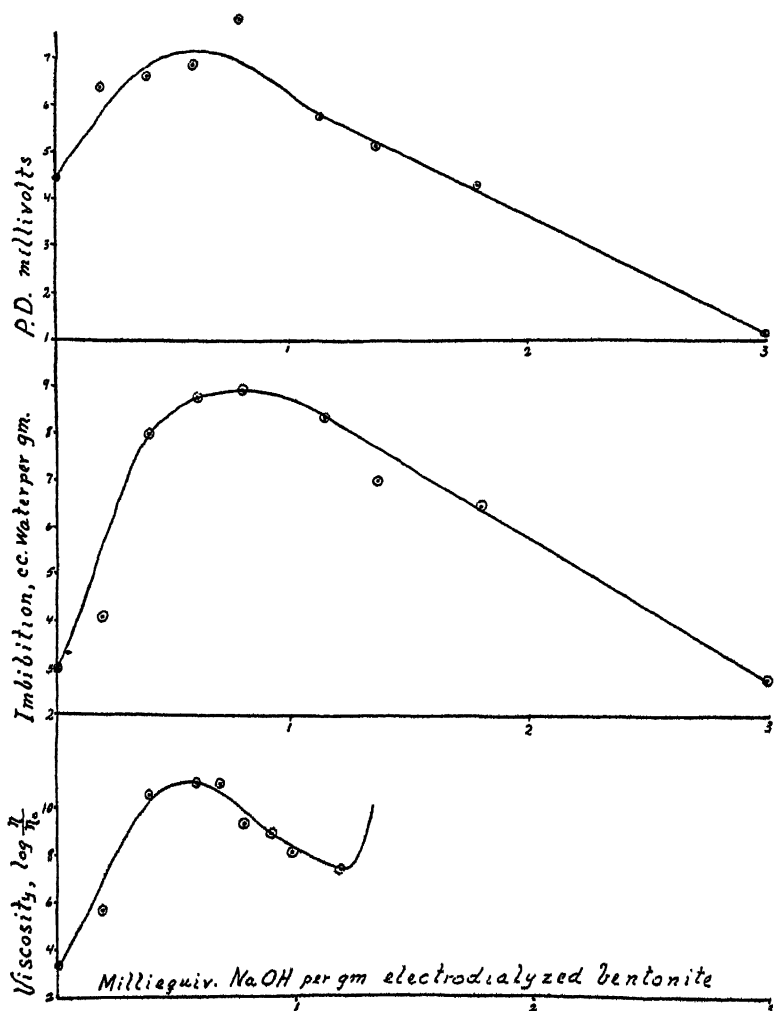


FIG. 3. THE INFLUENCE OF NaOH ON THE P.D., THE SWELLING AND THE VISCOSITY OF ELECTRODIALYZED BENTONITE

expressed views on the micellar structure. In the outer strata of the ion atmosphere the ion density, and hence the osmotic pressure, is very low. The osmotic tension or imbibing force is here correspondingly weak. The osmotically imbibed water is therefore less rigidly held and submits more readily to

shifts and displacements than the deeper layers of water nearer the surface of the particles where the osmotic tension is much greater.

This view on the nature of plasticity is further supported by the fact that clays with a high silica/sesquioxide ratio are more plastic than the clays in which this ratio is low, as was first observed by van Bemmelen (4) and later, in the case of soil colloids, by W. O. Robinson.³ The high-ratio clays contain a higher proportion of exchangeable cations, are more dissociated in water, and disperse and swell therefore more than the low-ratio clays. Further, the plasticity depends upon the nature of the exchangeable cation. The more highly dissociated the exchangeable cation the greater is the plastic range, as in the case of the Na-saturated colloid as compared to the Ca-saturated.

The liquefying effect of adding Na_2CO_3 to a mass of clay (a practice in the ceramic industry) is to be explained as in the case of the viscosity. The salt suppresses the osmotic pressure and hence the swelling of the individual micelles. Other electrolytes do the same, but in most cases the coagulating effect will counteract any liquefying effect. The Na_2CO_3 or silicate fulfill a double function: first, their own coagulating action being weak they precipitate the more powerfully coagulating ions; second, they suppress the volume of imbibed water by suppressing the osmotic pressure.

SWELLING, VISCOSITY, AND POTENTIAL DIFFERENCE

Figures 2 and 3 give a graphic representation of the influence of salts and of NaOH on the swelling, viscosity, and P.D. of the original and the electro-dialyzed bentonite, respectively.

The P.D. values in figure 2 are taken from tables 3, 5, and 6. The actual P.D. between the inside of the gel and the outside solution has not been determined, but the calculated P.D. is equal to $58 \log \frac{x}{y}$, x and y being observed values. The P.D. values in figure 3 are taken from table 13.

The swelling of bentonite in the different salt solutions as shown in figure 2 was determined separately by placing 0.2 gm. of the granulated material in graduated 10-cc. test tubes and carefully adding the different solutions by means of a pipette. The final volume occupied by the gel was recorded as the swelling. In the case of the electro-dialyzed bentonite to which NaOH in varying proportions was added, the swelling—in this case the amount of water imbibed per gram solid—as shown in figure 3 represents the $\frac{\text{water}}{\text{colloid}}$ ratio as given in table 13.

The viscosity curves in figures 2 and 3 were constructed from the viscosities as shown in tables 15 and 16, respectively. The ordinates represent the logarithm of the relative viscosities.

The results are here briefly recapitulated.

³ Unpublished.

The valence effect as shown by the curves in figure 2 is unmistakable. According to theory the P.D. should be more suppressed by the chloride than by the sulfate and should be least suppressed by the ferrocyanide. At the same concentration of the gel, that is, at the same z concentration, the P.D.'s should be in the ratio of 1.0; 1.33; and 1.60, respectively (comp. table 2). This relationship is very nearly approximated in the case of the chloride and the sulfate, the experiment with the ferrocyanide being less accurate as already explained.

Under conditions of free imbibition the valence effect expresses itself in a difference in swelling, the order in which the swelling is suppressed being the same as in the suppression of the P.D.

The descending parts of the viscosity curves show the same order. The increase in the viscosities in higher concentrations is due to the formation of aggregates (flocculation). Here again we meet with a valence effect of the anions. This represents a suppression of the stability of the suspensions. The chloride is the first to flocculate and causes accordingly a more rapid increase in the viscosity. The flocculating power of the sulfate comes next in order, that of the ferrocyanide being the weakest.

This leaves no doubt as to the influence of the valence of the ions having the same sign of charge as that of the colloid. This influence has been denied by Loeb who admits only an influence of the ion in combination with the colloid. Loeb apparently overlooked the fact that the equilibrium equation accounts for the influence of the valence of both ions, the influence being in opposite directions.

The curves in figure 3 show at first an increase in the P.D., the imbibition, and the viscosity as the electrodyalyzed material is being saturated with NaOH. This is accounted for by the formation of a highly dissociated NaOH saturated complex. The maxima coincide with the quantity of base which corresponds to the base exchange capacity. An excess of the base results in a suppression of the P.D., the swelling, and the viscosity, just as when any other electrolyte is added.

SUMMARY

Ions which do not enter into combination with, and are not positively adsorbed by the soil colloids were found to be negatively adsorbed, that is, the concentrations of the ions were found to be greater in the outside solution than in the solution within the gel after equilibrium was established. In the case of the Na-, K-, Ca-, and Ba-saturated colloids, the negative adsorption of the Cl ion in solutions of the respective chlorides was greatest in the Na-colloid-NaCl system and smallest in the Ba-colloid-BaCl₂ system, as shown by the series.



In the case of the NaCl -, NaNO_3 -, Na_2SO_4 -, and $\text{Na}_4\text{Fe}(\text{CN})_6$ -, Na-colloid systems the order of the negative adsorption of the anions is

$$\text{Cl} = \text{NO}_3 < \text{SO}_4 < \text{Fe}(\text{CN})_6$$

On the theory that a part of the exchangeable cations exist in a dissociated condition, the underlying principles of this relative order of ion distribution is easily understood. Surrounding the soil particle, i.e., in the micellar solution, there is an excess of cations. This must lead to an unequal distribution of the free electrolyte between the micellar and intermicellar solutions according to the laws of the Donnan equilibrium. The application of the equilibrium formulas on the valence effect showed a fair agreement between theoretical and observed differences.

Special attention was given to the valence effect of the anions, i.e., the ions of the same sign of charge as the colloid because of the denial of Loeb of any such effect. But it has been shown that the equilibrium equation demands, and that the experimental data prove a valence effect of these ions. This effect is opposite to that of the cations, i.e., the ions of opposite sign of charge to the colloid.

In the absence of free electrolyte the P.D., the swelling, and the viscosity are alone governed by the degree of dissociation of the exchangeable cations. The greater the dissociation the greater is the imbibition of water, which proceeds until there is an equilibrium between the osmotic and electrostatic forces. The dissociation depends upon the specific nature of the cations such as hydration and potential, and not merely on the valence. It is in this relationship that the ions arrange themselves in the order of the Hofmeister or lyotropic series.

In the presence of free electrolyte, the P.D., the swelling, and the viscosity are suppressed and this suppression depends solely on the valence of the ions in accordance with the thermodynamic and osmotic equilibrium equations. The Donnan equilibrium is to be dealt with as an effect and not as a cause. It does not create the aforementioned phenomena but is merely related to their suppression. All are effects of the same cause, namely, the formation of an ion atmosphere around a colloidal particle, i.e., the colloidal micelle.

When the gel is free to swell the valence effect shows itself in differences in the swelling or imbibition. If the swelling is inhibited, i.e., when the gel concentration is kept constant, the valence effect shows itself in differences in the negative adsorption, i.e., in the values of $\frac{x}{y}$ and in the P.D.

The colloidal micelle may be likened to a living organism. It reacts to any change in the surrounding medium only more quickly because it possesses no membrane. Remove the water and the micelle is destroyed leaving an inert particle comparable to the spore of an organism. This process is reversible. In dilute solutions (hypotonic) it swells, in strong solutions (hypertonic) it shrinks, thus striving to maintain a definite osmotic pressure and P.D. with respect to the outside solutions.

Distinction must be made between two forms of soil solution. The micellar solution is an integral part of the micelle. Any attempt to remove this solution by pressure or by any other means must fail.

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A STUDY OF DIASTASE ACTIVITY IN PLANTS: THE EFFECT OF PHOSPHATES IN THE SOIL MEDIA

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In an earlier paper (2) the results of a study of the effect of increasing amounts of potassium salts in the soil media upon the diastase activity of nasturtium plants were reported. No definite evidence of an expected correlation between the amount of potassium salt applied and activity was found but there appeared to be a correlation with favorable growing conditions, as was indicated by height, appearance, and dry weight data. Since a number of samples of soybeans which had been grown with a varying phosphate treatment were available¹ for study, it appeared of interest to determine the relative activity of these to see whether the correlation between favorable conditions for growth and diastase activity would be similarly evident under phosphate treatments.

PRODUCTION AND PREPARATION OF THE SOYBEAN MATERIAL

The plants were grown in the greenhouse in pot cultures. Mono-calcium and rock phosphates were applied at the rate indicated in table 1. The roots and tops were separated when cut and were dried in paper sacks hung in the greenhouse for about a month. The samples were ground and the phosphorus content and diastase activity determined.

DETERMINATION OF DIASTASE ACTIVITY

In the beginning an effort was made to use an extract of the soybean material, following the general procedure of Oshima (3), for the determination of diastase activity. However, when 2.5 gm. of the sample was extracted with 100 cc. of water for 3 hours a 70-cc. portion of the filtrate had a negligible action on starch solution. Accordingly, much the same method followed in the earlier paper was used.

PROCEDURE

A 0.5-gm. sample of the plant material was weighed into a small Erlenmeyer flask. From a pipette, 100 cc. of 2 per cent starch solution was introduced,

¹ The samples were grown, harvested, and analyzed for phosphorus by Mr. H. A. Lunt in connection with certain problems which he had under investigation. We are greatly indebted to him for his kindness in giving them to us for this study.

4 or 5 drops of toluene added, and the mixture shaken. At the same time a blank was prepared in the same way and immediately 10 cc. of 2*N* NaOH added. Digestion of the sample was allowed to proceed for 3 hours at 40°C., then the enzymatic action was stopped by adding 10 cc. of 2*N* NaOH. Apparent dextrose was determined on an aliquot portion by Defren's (1) method as in the earlier work. To insure the absence of particles of plant material a short piece of rubber tubing holding a small plug of absorbent cotton was

TABLE 1
Diastatic activity of soybean plant material grown in soil treated with varying amounts of phosphates

| SAMPLE | PHOSPHATE TREATMENT | TOPS | | | ACTIVITY | RATIO |
|-----------------------|------------------------|-----------------|---------------|--------------------|----------|-------|
| | | Green weight | Dry weight | Dried material | | |
| <i>Acid phosphate</i> | | | | | | |
| | <i>lbs. per acre</i> | <i>gm.</i> | <i>gm.</i> | <i>per cent P.</i> | | |
| 2 | No P | 20.5 | 5.8 | 0.076 | 1.241 | 100 |
| 3 | 5 | 38.5 | 10.6 | 0.090 | 1.487 | 120 |
| 4 | 5 | 42.0 | 12.0 | 0.088 | 1.426 | 115 |
| 7 | 15 | 91.0 | 27.7 | 0.096 | 1.244 | 100 |
| 8 | 15 | 98.5 | 28.5 | 0.096 | 1.307 | 105 |
| 9 | 15 | 90.0 | 27.0 | 0.106 | 1.303 | 106 |
| 5 | 25 | 111.0 | 33.0 | 1.128 | 1.359 | 110 |
| 6 | 25 | 123.5 | 36.5 | 0.120 | 1.430 | 115 |
| 10 | 150 | 158.0 | 44.5 | 0.293 | 1.187 | 96 |
| 11 | 150 | 147.0 | 40.0 | 0.316 | 1.025 | 83 |
| 12 | 150 | 166.0 | 45.7 | 0.332 | 0.961 | 77 |
| <i>Rock phosphate</i> | | | | | | |
| 13 | 500 R.P. | 20.5 | 5.7 | 0.062 | 1.451 | 101.0 |
| 14 | | 24.0 | 7.0 | 0.055 | 1.429 | 99.5 |
| 15 | | 21.0 | 6.4 | 0.052 | 1.439 | 100.0 |
| 16 | 2000 R.P. | 25.0 | 8.3 | 0.056 | 1.416 | 98.5 |
| 17 | | 28.0 | 8.2 | 0.069 | 1.461 | 101.5 |
| 18 | | 24.0 | 7.0 | 0.070 | 1.572 | 102.0 |

placed over the tip of the pipette while the aliquot was being withdrawn. Although the reducing value of the mixture of the hydrolytic products of starch is usually expressed as maltose, for comparative work of this type it makes no difference in terms of which sugar it is expressed. If desired, the "apparent" maltose can be calculated by use of its reducing ratio to glucose.

The grams of apparent dextrose which would have been produced by 1 gm. of the soybean material under the conditions of the experiment represents the "activity" of the sample.

The results of the determinations are given in table 1. To sample number 2 the no phosphorus treatment, is arbitrarily assigned an activity of 100 and the

acid phosphate samples are compared to it. In the rock phosphate series sample number 15, which has the lowest phosphorus content, is the standard of reference.

DISCUSSION OF RESULTS

In the table it is apparent that the amount of phosphorus taken up by the plant is roughly proportional to the amount of acid phosphate applied. The growth of the plants as indicated by dry weight production is also of this general agreement. Although the plants receiving the equivalent of 5-25 pounds of acid phosphate per acre tend to show a slight increase in diastase activity over the no phosphorus plants, the variations are not consistent and have no close relation with increase in dry weight. With the equivalent of 150 pounds of acid phosphate per acre, the activity falls off with an increase in the phosphorus taken in, even though the dry weight production still increases. This seems to indicate that diastase activity is not related to favorable conditions for growth alone but also to other factors. The decrease in activity and growth noted in the previous paper was undoubtedly due to over-feeding phenomena. A close relationship between diastase activity and potash nutrient is still probable. It appears that the high concentration of acid phosphate in the soil solution has a slight detrimental effect upon the elaboration of diastase, although small or moderate amounts may have a slight beneficial effect.

With the rock phosphate treatments the differences to be noted as regards phosphorus taken in, dry weight produced, and diastase activity are no greater than the experimental error.

SUMMARY

Soybeans were grown in the greenhouse in pot cultures with different amounts of acid phosphate fertilizers. Although the dry weight and phosphorus taken into the plant increased markedly with increasing applications of the fertilizer, the changes in diastase activity were slight for moderate amounts. With large amounts the activity seemed to fall off even though the dry weight and phosphorus taken in still increased.

With rock phosphate no significant differences in any respects were observed between the untreated and treated pots.

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A METHOD FOR THE DETERMINATION OF TOTAL CARBON AND ALSO FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS

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The titrimetric method has greatly simplified the estimation of carbon dioxide. Truog (12) has shown that the bead tower facilitates and hastens the absorption of carbon dioxide and that the single titration (8, 9, 13) is to be preferred to the double titration method (2, 3, 6, 7). A very simple trap for the absorption of carbon dioxide has been devised which combines the single titration with the use of the bead tower and sodium hydroxide. This trap and the method described may be used for the estimation of carbon dioxide from various sources, such as the carbon dioxide evolved from the soil by biological processes or from the combustion of organic materials by the wet method² (1). The details of the apparatus and its operation are given in this paper.

APPARATUS ADAPTED TO THE CHROMIC ACID METHOD FOR TOTAL CARBON³

The details of this apparatus are shown in figure 1. A 200-cc. Erlenmeyer flask (*D*) is closed with a 3-hole rubber stopper into which is fitted an inlet tube (*B*) leading from a soda-lime tube (*A*), a 12-inch water cooled condenser (*E*) with a rather small bore inner tube and a separatory funnel (*C*) through which the reagents are introduced. The inlet tube is so adjusted that it extends to a point near the bottom of the flask (*D*). The lower end of the condenser (*E*) is drawn out and a hole blown in the side so as to facilitate the free passage of gases with no interference from the condensed liquid.

The fume trap. At the top of the condenser (*E*) is fitted a U-tube (*F*) so arranged as to form a double trap to remove the chlorine and sulfuric acid fumes from the gas. This trap follows somewhat the plan of White and Holben

¹ From the Departments of Soils and Agricultural Bacteriology.

² There are indications that substances like alcohol or acetic acid are not completely oxidized by the wet method but are partially volatilized from the oxidizing solution.

³ While this paper was in press, an article appeared by T. E. Friedemann and A. I. Kendall [The determination of carbon and carbon dioxide. Jour. Biol. Chem. 82: 45-55 (1929)] in which a similar apparatus is described for the determination of total carbon and carbon dioxide. These authors also report the recovery of 99 to 100 per cent of the carbon as carbon dioxide from acetic acid and ethyl alcohol by the use of a high concentration of sulfuric acid in the oxidizing mixture.

(14). The bottom of the U-tube is filled with glass beads and above these in each arm is placed a small loose plug of glass wool. The first arm (*F1*) is filled with 20-mesh pumice saturated with a concentrated solution of silver sulfate, and the second arm (*F2*) with pumice saturated with concentrated sulfuric acid which has been boiled for two hours to remove the dissolved

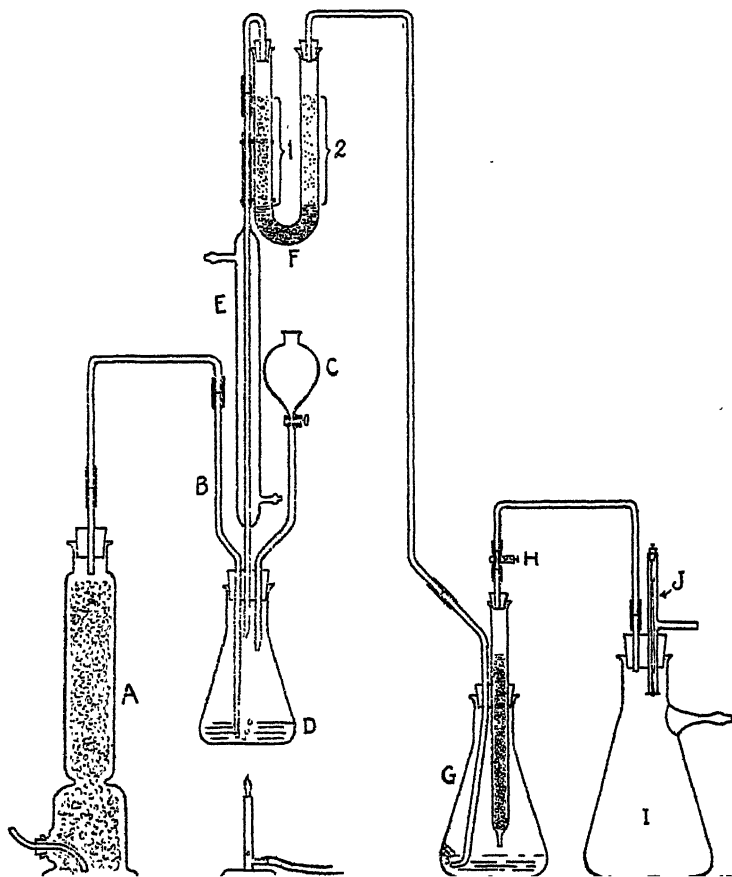


FIG. 1. APPARATUS, INCLUDING A NEW CARBON DIOXIDE TRAP, FOR THE DETERMINATION OF TOTAL CARBON BY WET COMBUSTION

gases. The silver sulfate removes the chlorine, and the sulfuric acid the sulfurous acid fumes. The contents of this trap are easily replaced and should not be used too long. This "dry" trap allows free passage of gases and removes all danger from back pressure which may be encountered with the wet trap.

The carbon dioxide trap. This trap (*G*) is adapted to the single titrimetric estimation of carbon dioxide and to the use of sodium hydroxide. It works on the same principle as the Truog (12) trap but is simpler and operates more

rapidly. The materials necessary for its construction are few and simple: a 300-cc. Erlenmeyer flask, a $\frac{3}{4}$ x 10 inch test tube, a number 6 rubber stopper, a little glass tubing, and a few glass beads. By means of a blast lamp, the test tube is drawn out at the bottom, and while still hot the upper part of the constriction is flattened so that two beads will lie at the bottom and allow a free passage of fluid either way. This point is important as it is necessary that all of the solution in the tower be easily washed down into the flask. The opening in the bottom of the tower should be large enough to allow rapid washing. Four- to six-millimeter solid glass beads are most satisfactory. The lower end of the entrance tube of the trap is drawn out and bent toward the side of the flask so that as the bubbles emerge from the tip they adhere for a time on the side of the flask before diffusion. The exit from the trap is made through the bead tower where the last traces of carbon dioxide are absorbed. This trap will handle gas flowing at the rate of 4 to 8 or even more bubbles per second, but usually a less rapid flow is more satisfactory. The rate of the gas flow is regulated by the screw-cock (*H*) and the extent of the vacuum by the valve (*J*). This valve is made by holding a thin rubber disk against the lower end of a glass T-tube by the aid of a light rubber band, two pins, and a small cork. By moving the upper pin up or down through the cork, the valve can be regulated for any desired pressure and the water in the suction pump may be allowed to run more rapidly than if the valve is not used. This valve will maintain a constant pressure and remove any danger of back suction even if the water pressure does vary somewhat.

Method of operation. The charge is placed in flask (*D*) which is then attached to the apparatus. The amount of material used is governed by the amount of carbon which it contains. From 20 to 100 mgm. of carbon are convenient amounts with which to work, but more may be used if necessary; the method is also accurate with smaller amounts. To the carbon dioxide trap (*G*) is added a measured quantity of 0.5 *N* carbon-dioxide-free sodium hydroxide. The amount added should be in excess of the amount necessary to absorb all of the carbon dioxide as sodium carbonate. The volume is made up to about 40 to 50 cc. with carbon-dioxide-free distilled water. On starting the flow of gas, the bead tower is allowed to dip into the solution until the liquid reaches the top of the beads. The tower is then raised and no more solution is allowed to enter. A little glycerol between the glass tower and the rubber stopper facilitates the adjustment of the tower. The flow is then regulated to about 3 to 5 bubbles per second. The oxidizing solutions are then introduced into the flask (*D*) through the separatory funnel (*C*). The oxidizing mixture is heated as rapidly as possible but not so as to cause it to back up into the inlet tube. A flow of air into flask (*D*) should always be maintained. The content of flask (*D*) is brought to boiling and boiled for 10 or 15 minutes or until all of the carbon dioxide has been driven off.

The carbon dioxide trap (*G*) is then detached and all of the alkali from the bead tower and entrance tube is washed into the flask with carbon-dioxide-free

distilled water, a supply of which may be obtained conveniently from an elevated carboy. An excess of neutral 2 *N* barium chloride is added and the excess alkali titrated with 0.5 *N* carbon-dioxide-free hydrochloric acid with phenolphthalein as the indicator. The difference between the amounts of alkali and acid used, multiplied by three, gives the milligrams of carbon. One cubic centimeter of 0.5 *N* sodium hydroxide is equivalent to 3 mgm. of carbon or 11 mgm. of carbon dioxide. For this method there should always be a blank determination so as to deduct the carbon dioxide in the apparatus and the reagents.

APPARATUS FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS

The evolution of carbon dioxide from a soil is one of the best indices of its biological activities. For the study of the respiration of soil microorganisms it is necessary to have a rapid and accurate method for estimating the carbon dioxide evolved. For this purpose, the carbon trap and method just described have been used to advantage in previous work (4). The equipment is inexpensive and simple in its construction, being made up of ordinary laboratory supplies, and the system allows itself to be added to until large numbers can be run at one time. Sets of 40 to 60 or more units can be aspirated at the same time from one small pump, and as many as 50 or more carbon dioxide determinations made in a 4-hour period.

Aspiration. Various methods of aspiration have been used for collecting carbon dioxide from soil. The aspiration may be either continuous or intermittent. When continuous, a steady flow of air at a rate of about one or two liters an hour is maintained through the aspiration chamber. With the intermittent system, aspiration is maintained for a few minutes once or twice a day and the chamber is closed for the remainder of the time. The intermittent system has the disadvantage of a heavy accumulation of carbon dioxide between aspirations. At times, in fact, this accumulation is so great that anaerobic conditions are approached and the activities of the microorganisms greatly inhibited. Under these conditions the carbon dioxide liberated for any given period may be lowered by one-half.

The aspiration may be directly over the soil (2) which is placed in a flask, or the air may be drawn through an aspiration chamber such as a bell jar or similar equipment in which a pot or beaker containing the soil is placed (8, 9, 10, 13). Other workers (3, 6, 7) have drawn the air directly through the soil which is placed in a cylinder or similar container. Aspiration over the soil permits the diffusion of carbon dioxide which would occur under normal field conditions, but when the air is drawn through the soil there is an abnormal soil atmosphere with either intermittent or continuous aspiration. Continuous aspiration through the soil gives too much oxygen and the intermittent system gives long intervals of diminished diffusion and high carbon dioxide concentration interspersed with one or two short daily intervals of high oxygen content.

It seems, therefore, that the most nearly normal and uniform soil atmosphere is maintained by a slow continuous aspiration of carbon-dioxide-free air over the soil. The carbon trap described is well adapted to this sort of aspiration. A very satisfactory continuous aspiration for large numbers of flasks is maintained by means of a small suction pump having a constant pressure valve (G, fig. 2) on the suction flask (F).

Operation. The details of the set-up for carbon dioxide evolution from soils are shown in figure 2. The air enters the scrubbing train through the soda-lime tube (A). If large numbers of determinations are being made at the same time, one or more soda-lime tubes may be added to care for the extra volume of air. Soda-lime is preferable to potassium hydroxide solution because it gives a freer passage of air with less danger of stoppage or back pressure. The air then goes to tower (B), which is filled with coarsely granulated pumice saturated with

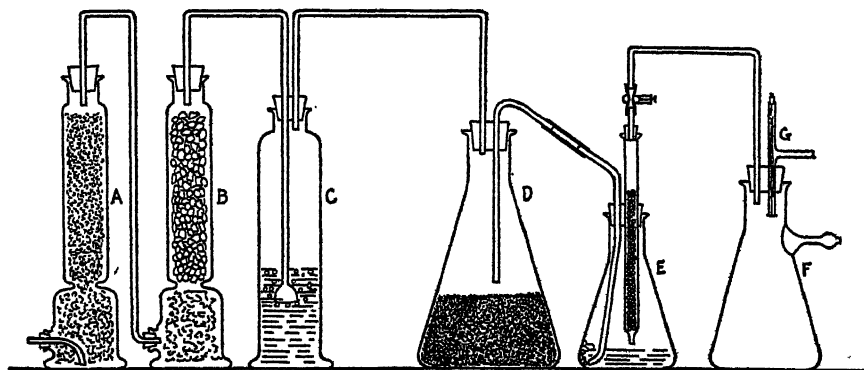


FIG. 2. APPARATUS FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM A SOIL

sulfuric acid (dilute 1-2). This tower removes the ammonia. Tower (C) is partly filled with a dilute solution of barium hydroxide and the air is bubbled through this solution. The prime purpose of this tower is to saturate the atmosphere with moisture so that as much moisture is carried into the soil flask as is carried out of it. By this means a 600-gm. sample of soil has been carried for a month without loss of moisture. The barium hydroxide is introduced into tower (C) to serve as an indicator in case the contents of tower (A) become spent and allow the carbon dioxide to pass. At the same time it acts as an emergency carbon dioxide trap in the scrubbing train. Two units of (C) may be used if desired.

The carbon-dioxide-free air, now saturated with moisture, enters the soil flask (D) at the top and the carbon-dioxide-laden air is drawn off just above the soil surface. It is well not to have the outlet tube nearer to the soil than about one-half inch, as drops of moisture will sometimes collect at the bottom

of the tube and if these drops touch the soil, stoppage may occur. The soil containers used in this case were 750-cc. Erlenmeyer flasks with 600 gm. of soil, but this is a matter of adjustment to suit conditions. The carbon-dioxide-laden air passes into the carbon dioxide trap (*E*) where the carbon dioxide is absorbed. At the end of the aspiration period the traps are removed in pairs and the carbon dioxide determined as already outlined.

DISCUSSION

The use of sodium hydroxide in place of barium hydroxide has three distinct advantages: (a) The solution may be more concentrated; (b) the carbonate

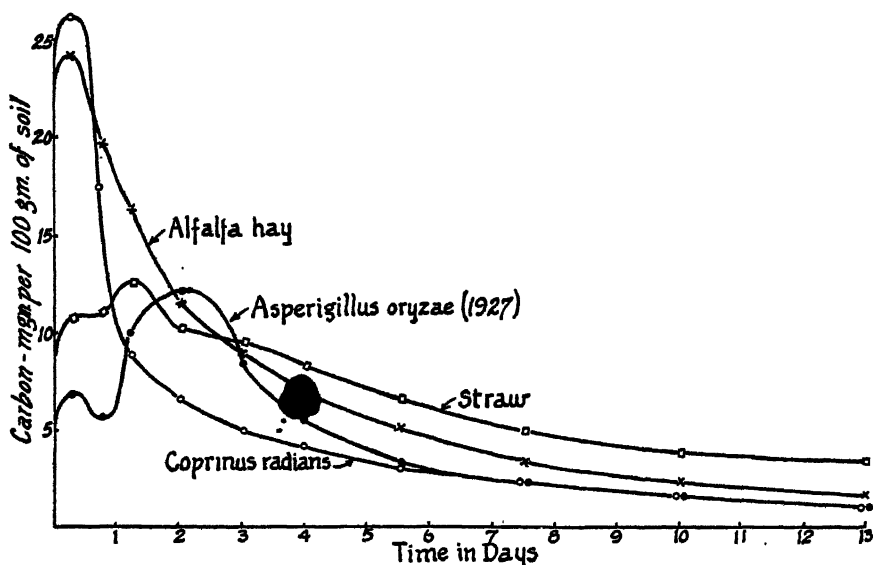


FIG. 3. RATE OF EVOLUTION OF CARBON DIOXIDE FROM VARIOUS SUBSTANCES

The amounts of organic materials varied but each substance contained 10 mgm. of nitrogen

does not precipitate and the solution in the bead tower can be washed out easily with water without removing the beads; and (c) if insufficient sodium hydroxide is used in the trap to absorb all of the carbon dioxide as the carbonate, some will be absorbed as the bicarbonate, thus increasing the capacity of the absorbing solution. Ledig (5) has shown that potassium hydroxide absorbs carbon dioxide more rapidly than does sodium hydroxide, but it was found in this work that sodium hydroxide gave perfect absorption. Carbon-dioxide-free sodium hydroxide is also more easily prepared than the carbon-dioxide-free potassium hydroxide because sodium carbonate is less soluble than potassium carbonate.

In the presence of large amounts of barium carbonate the color change of phenolphthalein is not so sharp as one would like, and the titration should not

be hurried especially on nearing the end point. With white artificial light or good day light, the titration should be carried on until the pink color has entirely disappeared. Schollenberger (11) recommends thymolphthalein in place of phenolphthalein because of the higher pH value of its end point. The author, however, prefers phenolphthalein, with which an accuracy of from 0.1 to 0.4 mgm. of carbon may easily be obtained.

Plate 1 shows 42 flasks and carbon traps operating on one pump and attached to one scrubbing train. The rate of evolution of carbon dioxide from organic materials placed in the soil is shown in figure 3. During the early periods when the carbon dioxide evolution is high, the intervals between analyses may be as short as 8 or 12 hours, and as the evolution decreases the intervals may be lengthened to several days or a week if desired.

SUMMARY

A rapid method for the estimation of carbon dioxide is given. This method is applicable to the determination of organic carbon, carbonate carbon, or the carbon evolved from a soil as carbon dioxide. A total carbon determination can be made in about 45 minutes and carbon dioxide determinations at the rate of about 10 to 20 an hour.

A simple carbon dioxide trap is described which combines the bead tower and the use of sodium hydroxide with the single titration principle. The carbon dioxide is absorbed in 0.5 *N* sodium hydroxide solution and precipitated as the carbonate by the addition of an excess of 2 *N* neutral barium chloride. The excess alkali is then titrated with 0.5 *N* hydrochloric acid, phenolphthalein being used as the indicator. The difference between the cubic centimeters of standard alkali and acid used, multiplied by 3, gives the milligrams of carbon.

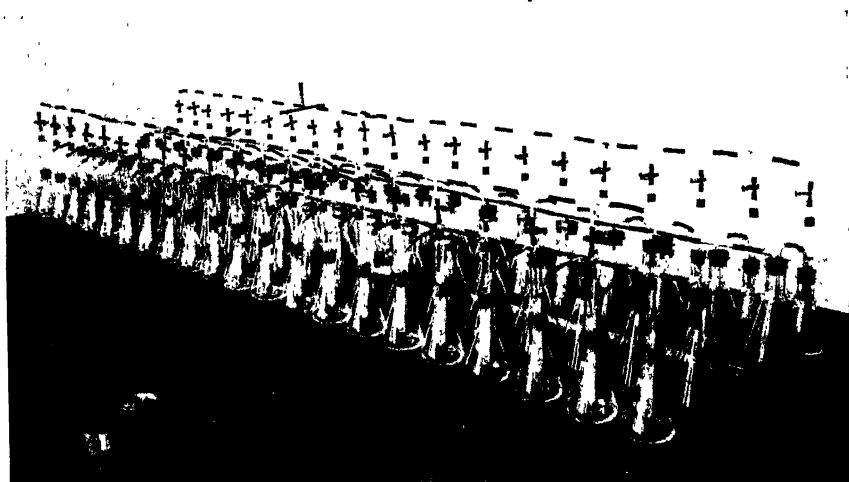
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PLATE 1

SET-UP USED IN CARBON DIOXIDE EVOLUTION FROM SOILS



COLLOIDAL PROPERTIES OF WILLAMETTE VALLEY SOILS

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A very much more definite knowledge of the meaning of acid and alkali soils has resulted from increased knowledge of the colloidal fraction of the soil. The retention of essential nutrient anions by the soil is much better understood since recent developments in the field of soil colloids. Though there is a tendency to overemphasize certain phases of work at certain times, it seems reasonable to assume from our present knowledge, that most of the chemical and physical properties of soils are in some way connected rather intimately with the amount and condition of the colloidal fraction. Knowledge of the colloidal properties of soils is therefore of great practical and scientific interest. This study was planned especially to observe correlations between chemical and physical properties of soils and their colloidal components.

PLAN OF STUDY

The following nine soils were selected for study: Chehalis Fine Sandy Loam, Cove Clay, Willamette Silty Clay Loam, Dayton Silty Clay Loam, Aiken Clay Loam, Olympic Clay, Melbourne Silty Clay Loam, Peat, and Muck. All soils except the peat and muck were sampled by horizons, so that each horizon of the profile could be studied separately.

Two of these soil series, Chehalis and Cove, are recent formations. The Chehalis is a free working, well-drained and highly productive soil. The Cove on the other hand is very heavy and tight, difficult to till, but fertile when once in crop.

Two series, the Willamette and Dayton, are old valley-filling or high terrace formations. The Willamette is a well-drained, highly productive, and desirable agricultural soil. The Dayton on the other hand is poorly drained, and underlaid with a sticky blue clay that makes it difficult to till and rather undesirable as an agricultural soil.

Two series, Aiken and Olympic, are residual hill soils from igneous rocks, largely the product of the weathering of basalt. Both are rather desirable agricultural soils, with good drainage, and are fairly fertile when well farmed. The chief outward distinction is the red color of the Aiken in contrast to the brown color of the Olympic.

The Melbourne series is likewise a residual hill soil, but is formed from

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sedimentary instead of igneous rock. The underlying sandstones are sometimes rather close to the surface, but on the whole the Melbourne is a desirable soil.

Two organic soils, a Lake Labish Peat and a Clackamas County Muck, were included in part of the studies to observe characteristics which might be related to their organic nature. The soils were sampled and stored outdoors in large jars to maintain a moist condition throughout the period of study.

The study included a determination of the amount of colloid in the different horizons of all soils, together with a study of the chemical composition of both the colloid and the whole soil. For this phase of the work, iron, aluminum, silica, and calcium were determined so that any relations which might exist between composition and physical properties might be observed. There was included also a study of exchangeable calcium, and the retention of one anion, PO_4 , by the colloids and the whole soils.

Because of the laboriousness of the task and the limitations of time, the colloidal material separated from the three horizons of only one soil series, the Chehalis. The data are therefore inadequate for definite conclusions at the present time.

EXPERIMENTAL

Isolation of colloids

The definition for "soil colloid" is somewhat arbitrary. The Bureau of Soils (7, 8) defines the colloidal material on the basis of the size of particles and selects one micron as the upper limit for colloidal size. Their method for separating the colloid from coarser material was employed except that fresh moist soils were used and the period of sedimentation was 10 days, as recommended by Bradfield (3). After passing through the supercentrifuge once the colloidal suspension was deep amber in color, and contained a little more than 0.3 per cent solid matter. After a second centrifuging the suspensions were lighter in color and contained about 0.05 per cent of solid matter. When filtered on the Pasteur-Chamberlain filter, a clear filtrate resulted, and the colloidal mass remaining on the candles was a reddish amber, jelly-like mass. When dry the jels formed hard, horny, brittle masses.

Properties of colloids

Largely because of lack of suitable methods for accurate determinations, early workers often placed the quantity of colloidal material in soils at no more than 0.5 to 2 per cent.

The chief difficulty lay in the practical impossibility of dispersion of colloidal material and therefore the inability to separate any appreciable amount by sedimentation methods. Since indirect methods have been devised, based upon specific properties of colloids, more reliable information has been obtained and much larger amounts of colloid are found to be present in the average good

soil. After trying various methods the water vapor absorption method was found fully as satisfactory as any by Gile et al. (8).

The water vapor method was used in the following study, practically as planned by them (8, 9). Since the colloidal material was separated from the three horizons of only one soil, the Chehalis, only for these three colloids could

TABLE 1
*Amount of colloid found by the water vapor method**

| SOIL TYPE | HORIZON | PER CENT COLLOID |
|---------------------------------|---------|------------------|
| Chehalis Fine Sandy Loam..... | I | 22.8 |
| | II | 25.7 |
| | III | 25.1 |
| Cove Clay..... | I | 42.7 |
| | II | 65.8 |
| | III | 62.4 |
| Willamette Silty Clay Loam..... | I | 20.7 |
| | II | 20.2 |
| | III | 29.7 |
| Dayton Silty Clay Loam..... | I | 25.3 |
| | II | 42.2 |
| | III | 51.1 |
| Aiken Clay Loam..... | I | 39.5 |
| | II | 42.5 |
| | III | 51.8 |
| Olympic Clay..... | I | 50.8 |
| | II | 55.9 |
| | III | 58.0 |
| Melbourne Silty Clay Loam..... | I | 30.2 |
| | II | 42.8 |
| Peat..... | | 95.6 |
| Muck..... | | 71.4 |

* Most of the analytical data in this paper was supplied by T. M. Tieh, a graduate student in the department of soils.

the specific absorption be determined. It was found to be for the surface horizon 0.263, subsoil 0.283, and parent material 0.284. Robinson (9) working with 34 colloids from different soils reports a range from 0.240 to 0.348. The mean specific absorption was 0.298.

In this work the mean specific absorption of 0.298 obtained by Robinson (9) was used as a basis for calculation in all determinations except for the Chehalis soil, for which the specific absorption was determined.

Table 1 shows the amount of colloid found in the various horizons of the different soils by this method.

It will be observed that the amount of colloid is rather large but correlates fairly closely with field texture. Robinson's data (9) show that percentage of colloid is rather uniformly higher in the subsoil, which under natural conditions in mature soils is heavier in texture than the surface. Out of 15 soils studied by Robinson (9), 13 show more colloid in the subsoil.

In every case except the Willamette in this study, there is appreciably more colloid in the subsoil. There may be a question whether the profiles as found are the result of maturity, or simply the result of deposition. Why the deep formation or parent material should show more colloid than is found in the

TABLE 2
Composition of soil colloid

| SOIL TYPE AND HORIZON | ORGANIC MATTER | SiO ₂ | Al ₂ O ₃ | Fe ₂ O ₃ | CaO |
|--|-------------------|------------------|--------------------------------|--------------------------------|-----------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Chehalis..... { I | 3.72 | 40.01 | 24.80 | 11.37 | 1.48 |
| II | 3.60 | 38.37 | 23.39 | 10.48 | 1.48 |
| III | 2.40 | 41.21 | 25.01 | 12.00 | 1.51 |
| Average of 45 soils—Bureau of Soils (10)..... | 4.02 | 43.34 | 26.83 | 10.70 | 1.05 |

TABLE 3
Relation of composition of colloids

| CHEHALIS HORIZON | SiO ₂ Al ₂ O ₃ + Fe ₂ O ₃ | CaO Al ₂ O ₃ + Fe ₂ O ₃ × 10 |
|------------------|---|---|
| | | |
| I | 2.11 | 0.84 |
| II | 2.16 | 0.89 |
| III | 2.11 | 0.84 |

subsoil can not be fully explained. Both organic soils necessarily show a high colloid content, since organic matter in the humified condition and even in the raw state, is colloidal in nature.

Chemical composition of soils and colloids

The data in table 2 in comparison with that in table 4 show that the colloidal material is higher in organic matter, lower in silica, somewhat higher in aluminum, and considerably higher in iron, but much lower in calcium than the whole soil. The percentage of total calcium in the whole soil is unusually high, however. When the data are compared with the average of 45 determinations by the Bureau of Soils (10) as shown in table 2, the results for the colloidal material are found to correspond closely.

Various workers have noted a correlation between the properties of soils and the chemical composition of the colloidal material (1, 6) especially when the ratio

$$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3} \text{ was studied and compared with the ratio } \frac{\text{CaO}}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}.$$

TABLE 4
Comparison of the soils

| SOILS | | ORGANIC MATTER | SiO ₂ | Al ₂ O ₃ | Fe ₂ O ₃ | CaO |
|------------|----------|-------------------|------------------|--------------------------------|--------------------------------|----------|
| | | per cent | per cent | per cent | per cent | per cent |
| Chehalis | I..... | 1.99 | 55.64 | 21.08 | 8.37 | 5.77 |
| | II..... | 2.09 | 55.12 | 22.87 | 8.28 | 6.00 |
| | III..... | 1.25 | 55.63 | 20.49 | 9.09 | 5.38 |
| Cove | I..... | 4.87 | 51.85 | 19.37 | 12.92 | 3.19 |
| | II..... | 0.94 | 52.81 | 21.99 | 13.54 | 2.86 |
| | III..... | 0.60 | 53.52 | 19.23 | 14.13 | 4.13 |
| Willamette | I..... | 3.91 | 63.41 | 12.15 | 10.89 | 1.88 |
| | II..... | 1.54 | 66.23 | 16.43 | 8.43 | 1.75 |
| | III..... | 0.79 | 63.18 | 13.11 | 13.64 | 3.39 |
| Dayton | I..... | 3.70 | 66.84 | 17.55 | 5.49 | 2.62 |
| | II..... | 1.03 | 66.12 | 16.71 | 6.91 | 1.97 |
| | III..... | 0.38 | 59.48 | 17.97 | 7.92 | 2.82 |
| Aiken | I..... | 3.07 | 47.15 | 22.80 | 16.74 | 0.66 |
| | II..... | 1.78 | 47.28 | 21.83 | 17.52 | 0.39 |
| | III..... | 1.03 | 55.99 | 24.14 | 18.44 | 0.48 |
| Olympic | I..... | 3.50 | 46.54 | 18.77 | 18.32 | 4.23 |
| | II..... | 1.70 | 49.97 | 19.09 | 17.79 | 3.44 |
| | III..... | 1.18 | 46.91 | 12.47 | 22.28 | 3.20 |
| Melbourne | I..... | 3.26 | 64.30 | 17.91 | 8.55 | 0.61 |
| | II..... | 1.39 | 61.92 | 18.81 | 8.46 | 0.80 |
| Peat..... | | 72.63 | | | | |
| Muck..... | | 46.10 | | | | |

The data in table 3 are too limited for study except by comparison with other available data. By comparing with results obtained by the Bureau of Soils (6) it is found that the ratio noted in table 3 places this soil in an intermediate group. The highest ratio reported by Gile (6) for aluminum and iron to silica was 3.62 while the lowest was 0.54. The ratio noted in table 3 is 2.11 with very little difference in the various horizons. The same may be said for the molecular ratio of aluminum and iron to calcium. The highest ratio reported

by Gile is 1.81 and the lowest 0.18. The ratio found here is 0.84 with little variation in the different horizons.

In a study of humid-tropical and humid-temperate soils Bennett (2) found close correlation between the physical properties of the whole soils and their chemical composition. On the basis of physical behavior as correlated to chemical composition he made two groups; namely, friable soils in which the ratio of $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3} =$ less than 2, and plastic soils in which the ratio was greater than 2.

The average of 24 friable tropical soils showed 29.30 per cent SiO_2 ; 14.91 per cent Fe_2O_3 ; and 34.28 per cent Al_2O_3 , with 0.24 per cent of calcium. Other soil bases averaged correspondingly low. These are lateritic soils.

TABLE 5
Relation of composition of soils

| HORIZONS | $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ | $\frac{\text{CaO}}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3} \times 10$ |
|-------------------|--|--|
| Chehalis I..... | 3.57 | 3.98 |
| Cove I..... | 2.97 | 2.10 |
| Willamette I..... | 5.62 | 1.79 |
| Dayton I..... | 5.38 | 2.27 |
| Aiken I..... | 2.38 | 0.36 |
| Olympic I..... | 2.59 | 2.50 |
| Melbourne I..... | 4.66 | 0.48 |
| Average..... | 3.88 | 1.92 |

The average for 24 non-friable tropical soils was 52.33 per cent SiO_2 ; 9.53 per cent Fe_2O_3 ; and 21.28 per cent Al_2O_3 ; and 1.55 per cent CaO, with other bases correspondingly higher than in the friable group.

The data in table 4 in comparison with these figures show that the soils under study resemble the non-friable group in composition to a much greater extent than the friable group.

The friable group is described as lateritic, very porous, deep, and uniform, high in colloidal content yet flocculated and granular. Though the data presented here are far too meager for definite conclusions, the two hill soils, Aiken and Olympic, show only slight lateritic tendencies in their slightly lower SiO_2 content and higher Fe_2O_3 content. These soils are somewhat friable, and inclined to good to excessive drainage.

The SiO_2 of all other samples was about as high as, and in most cases much higher than even that of the non-friable group, studied by Bennett. The Al_2O_3 and Fe_2O_3 contents, likewise, place most of the other soils decidedly in the non-friable group. Exceptions are the somewhat lower iron content of the Willamette and the somewhat lower silica content of the Cove series. These ex-

ceptions, however, do not appear significant. The general physical properties of these soils place them decidedly in the non-friable group.

The molecular ratios in table 5 show that in no case is the ratio $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ less than 2, but is closest to it in the Aiken and Olympic series. The ratio found by Bennett (2) for the average of the friable group was 1.25 while for the plastic group it was 3.71. In only one of these soils, the Aiken, does the ratio $\frac{\text{CaO}}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ appear to be especially low. It is rather low also in the Melbourne series, but with only meager data such variations are not necessarily typical.

Study of the different horizons of the various soils does not bring out any striking characteristics. The recent soils, as might be predicted, are rather more uniform in the composition of the different horizons than the older, more mature soils. With the exception of the Chehalis series, most of the organic matter is found in the surface horizons. In spite of leaching there remains as much lime in the surface as in any horizon in most cases.

There is noticeable absence of correlation between color and composition. Aiken, because of its bright red color, is locally known as a red-hill soil. Olympic on the other hand, which is a rich brown, contains slightly more iron. Cove, which appears rather uniformly black in color as deeply as it was sampled, contains five times as much organic matter in the surface horizon.

Base exchange

Exchangeable calcium was studied on the colloidal material from the different soil horizons and on the different horizons of all the soil series. These data are compared with the total calcium and with the reaction of the soil (table 6).

It is quite noticeable that a very large percentage of the calcium of the colloidal material is exchangeable whereas a rather small percentage of the calcium of the soil as a whole is exchangeable. This corroborates the oft made claim that the exchangeable and easily available calcium is practically all held by the colloidal complex, and that good soils must be abundantly supplied with this nutrient so held.

Aiken and Melbourne which have the lowest total exchangeable calcium have the highest percentage of total calcium in exchangeable form, indicating the important function of colloidal material in conserving the supply of available calcium.

It is noticeable also that the two soils which have the highest content of colloid (Cove and Olympic) also have the highest percentage of exchangeable calcium. This is not necessarily true, however, when severe leaching has occurred. None of these soils are very acid and are not therefore extremely leached.

Retention of anions

The manner in which available anions are held in soils has not been fully determined. The phosphate ion has been most studied. Russell and Prescott (12) believe that physical adsorption occurs, since their data can be

TABLE 6
Exchangeable calcium

| SAMPLES | | pH | EXCHANGEABLE CALCIUM | PROPORTION OF TOTAL CALCIUM EXCHANGEABLE |
|-------------------|----------|------|-------------------------|--|
| | | | <i>per cent</i> | <i>per cent</i> |
| Chehalis Colloids | | | | |
| Horizon | I..... | 5.11 | 0.98 | 92.5 |
| | II..... | 5.60 | 0.89 | 84.8 |
| | III..... | 6.00 | 1.07 | 99.1 |
| Chehalis Soil | I..... | 5.48 | 0.34 | 8.3 |
| | II..... | 5.51 | 0.40 | 9.3 |
| | III..... | 5.51 | 0.41 | 10.6 |
| Cove | I..... | 5.61 | 0.46 | 20.2 |
| | II..... | 6.14 | 0.58 | 28.3 |
| | III..... | 6.12 | 0.65 | 22.0 |
| Willamette | I..... | 5.36 | 0.35 | 24.5 |
| | II..... | 5.36 | 0.24 | 19.2 |
| | III..... | 6.63 | 0.36 | 14.8 |
| Dayton | I..... | 5.48 | 0.28 | 15.0 |
| | II..... | 5.41 | 0.31 | 22.0 |
| | III..... | 5.95 | 0.47 | 23.3 |
| Aiken | I..... | 5.11 | 0.16 | 34.0 |
| | II..... | 5.09 | 0.14 | 50.0 |
| | III..... | 5.02 | 0.15 | 42.9 |
| Olympic | I..... | 5.26 | 0.55 | 18.2 |
| | II..... | 5.55 | 0.71 | 28.9 |
| | III..... | 5.78 | 0.67 | 29.4 |
| Melbourne | I..... | 6.14 | 0.25 | 56.8 |
| | II..... | 5.73 | 0.24 | 42.1 |

adapted to Freundlich's Adsorption Isotherm. Comber (4), Fisher (5), and Teakle (13), however, explain it as purely chemical precipitation. Roszman (11) thinks that organic matter may play an important part in taking up soluble phosphates. Other important anions are not taken up in as large quantities as the phosphate.

Retention of PO_4

The retention of the phosphate ion by both the soils and the separated colloids was studied. The ratio of the weight of the samples and the volume of solutions was kept at 1 to 10. The solutions used were made up from di-sodium phosphate and mono-potassium phosphate to give approximately neutral mixtures of the different phosphate concentrations.

The soils and the phosphate mixtures were allowed to stand in contact for three hours, with occasional stirrings. A little sodium chloride was then added as a flocculent, and in a few minutes aliquots of the clear supernatant solutions were taken for analysis.

The data show a large amount of phosphate retained by the colloid material. At the lower concentrations, retention is nearly complete. When retention amounted to around 5000 p.p.m., however, the colloid material seems to have been approaching its approximate capacity.

TABLE 7
Amount of PO_4 taken up by colloids

| SOILS | CONCENTRATION OF PO_4 IN SOLUTIONS USED | | | | | | | |
|-------------------|---|--------|--------|--------|--------|--------|--------|--------|
| | 20 | 40 | 80 | 160 | 320 | 640 | 960 | 1280 |
| | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. |
| Chehalis Colloids | | | | | | | | |
| Horizon { I..... | 193 | 388 | 780 | 1,550 | 2,997 | 4,992 | 5,020 | 5,650 |
| II..... | 196 | 396 | 790 | 1,581 | 3,012 | 4,960 | 4,950 | 5,350 |
| III..... | 193 | 395 | 783 | 1,564 | 3,100 | 5,088 | 5,015 | 5,200 |

The curve for retention is a straight line up to 1550 p.p.m. at which point there is a slight break followed by a slight bend, and finally the curve again straightens.

Assuming that all exchangeable calcium found in the colloidal material should function to precipitate PO_4 ions, only about a third of the phosphate actually retained could be accounted for. Even after liberal allowance is made for the action of soluble iron and aluminum in precipitating phosphate there would yet remain a large amount not accounted for by chemical precipitation.

It seems probable, therefore, that the break in the curve may represent the point at which retention by chemical precipitation has been satisfied. Beyond this point retention is probably principally mechanical absorption in the interstitial spaces of the colloid matter. Phosphate so held should be rather easily removed from the soil.

Wiley and Gordon (14) found appreciable amounts of phosphate retained by silica gel, apparently a mechanical retention of the phosphate in the small pore spaces of the colloid. This retained phosphate could be washed out but with some difficulty.

Table 7 indicates that the soil as a whole has a much lower capacity for retention of phosphate than the colloidal material. The correlation between phosphate retention and percentage of colloid in the soil is not close however. Since the amount of the precipitating ions, of which calcium is doubtless very

TABLE 8
Amount of PO_4 taken up by soils from the following

| SOILS | CONCENTRATION OF PO_4 IN SOLUTIONS USED | | | | | | | |
|---------------------|---|--------|--------|--------|--------|--------|--------|--------|
| | 20 | 40 | 80 | 160 | 320 | 640 | 960 | 1280 |
| | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. | p.p.m. |
| Chehalis | | | | | | | | |
| Horizon { I..... | 150 | 289 | 456 | 503 | 1,120 | 1,440 | 1,872 | 2,104 |
| II..... | 165 | 309 | 452 | 598 | 1,286 | 1,984 | 2,100 | 2,100 |
| III..... | 180 | 339 | 473 | 600 | 1,125 | 1,716 | 1,800 | 2,097 |
| Cove { I..... | 191 | 383 | 603 | 920 | 1,762 | 1,880 | 2,052 | 2,250 |
| II..... | 193 | 384 | 705 | 960 | 1,660 | 1,600 | 1,900 | 2,048 |
| III..... | 194 | 372 | 635 | 880 | 1,441 | 1,472 | 1,953 | 1,850 |
| Willamette { I..... | 152 | 305 | 512 | 682 | 1,362 | 1,664 | 1,500 | 1,508 |
| II..... | 178 | 336 | 548 | 566 | 1,330 | 1,696 | 1,632 | 1,203 |
| III..... | 186 | 342 | 536 | 675 | 1,230 | 1,816 | 1,660 | 1,885 |
| Dayton { I..... | 169 | 313 | 512 | 682 | 1,180 | 1,408 | 1,550 | 1,550 |
| II..... | 190 | 348 | 548 | 740 | 1,488 | 1,792 | 1,752 | 2,050 |
| III..... | 188 | 342 | 536 | 720 | 1,444 | 1,748 | 1,648 | 1,813 |
| Aiken { I..... | 194 | 392 | 756 | 1,120 | 2,233 | 3,520 | 3,250 | 3,148 |
| II..... | 197 | 394 | 786 | 1,263 | 2,322 | 3,328 | 3,600 | 3,873 |
| III..... | 198 | 396 | 783 | 1,245 | 2,351 | 3,776 | 3,842 | 3,952 |
| Olympic { I..... | 191 | 380 | 655 | 905 | 1,708 | 2,816 | 2,652 | 2,950 |
| II..... | 188 | 397 | 655 | 878 | 1,600 | 2,400 | 2,450 | 2,160 |
| III..... | 186 | 360 | 604 | 773 | 1,536 | 2,272 | 2,355 | 2,280 |
| Melbourne { I..... | 193 | 388 | 608 | 974 | 1,662 | 2,528 | 2,350 | 2,200 |
| II..... | 197 | 393 | 650 | 1,018 | 1,400 | 2,560 | 2,308 | 2,402 |
| Peat..... | 158 | 355 | 645 | 980 | 1,931 | 2,240 | 2,650 | 2,888 |
| Muck..... | 196 | 392 | 873 | 1,382 | 2,868 | 2,960 | 4,250 | 4,900 |

important, varies considerably, lack of close correlation of phosphate retention with amount of colloid or any other single factor is not illogical.

From the above data it appears that organic matter in the peat soil is not especially effective in retaining phosphate. Muck which contains considerable mineral matter has a much higher retentive capacity. There is also a decided lack of correlation between phosphate retention and the percentage of

exchangeable calcium in the various soils, indicating again that precipitation of calcium phosphate is not the only important factor in phosphate retention. Other precipitating ions, the quantity of which is not known, are undoubtedly important factors in the phosphate retention. Some of the soils were rather high in iron and aluminum but how much came into the solution to combine with phosphate is not known.

TABLE 9
Available phosphate, phosphate retention, and the R_2O_3/SiO_2 ratio

| SOILS | | PO ₄ SOLUBLE IN 0.001 N H ₂ SO ₄ | PO ₄ RETENTION FROM SOLUTION CONTAINING 1280 P.P.M. PO ₄ | MOL. R ₂ O ₃ MOL. SiO ₂ |
|------------|----------|--|---|---|
| | | p.p.m. | per cent | |
| Chehalis | I..... | 1.0 | 0.21 | 0.28 |
| | II..... | 0.5 | 0.21 | 0.30 |
| | III..... | 0.3 | 0.21 | 0.28 |
| Cove | I..... | 0.4 | 0.23 | 0.31 |
| | II..... | 0.3 | 0.21 | 0.34 |
| | III..... | 0.2 | 0.19 | 0.31 |
| Willamette | I..... | 2.8 | 0.15 | 0.18 |
| | II..... | 0.5 | 0.12 | 0.19 |
| | III..... | 0.3 | 0.19 | 0.20 |
| Dayton | I..... | 0.9 | 0.16 | 0.19 |
| | II..... | 0.2 | 0.21 | 0.19 |
| | III..... | 0.2 | 0.18 | 0.23 |
| Aiken | I..... | 0.2 | 0.32 | 0.42 |
| | II..... | Trace | 0.33 | 0.41 |
| | III..... | Trace | 0.23 | 0.38 |
| Olympic | I..... | 0.3 | 0.30 | 0.39 |
| | II..... | 0.2 | 0.22 | 0.36 |
| | III..... | 0.2 | 0.23 | 0.34 |
| Melbourne | I..... | 0.2 | 0.22 | 0.21 |
| | II..... | 0.2 | 0.24 | 0.23 |

A study of the amount of phosphate (table 9), soluble in 0.001 *N* sulfuric acid in the untreated soils indicates a normally low availability. There appears to be no connection between the soluble phosphate in the soils and the retention of additional soluble phosphates. There does, however, appear to be some slight connection between soluble phosphate and the molecular ratio R_2O_3/SiO_2 . The Aiken and Olympic soils, which have a high ratio, show a low concentration of soluble phosphate, indicating that iron and aluminum may function

in rendering the native phosphates of the soil insoluble. This may be taken as some indication that the high ratio R_2O_3/SiO_2 may be correlated likewise to some extent with retention of the added soluble phosphate.

Regardless of what the specific relation may be, it is apparent that these soils have a high retentive capacity for the phosphate ion, and that this retentive capacity is rather intimately related to the colloidal fraction. It may likewise be concluded that little loss of soluble phosphates is likely to occur, even when the phosphates are added liberally to highly colloidal soils.

SUMMARY

1. The colloid content of nine soil series was determined by horizons and the colloid separated from each of the three horizons of one series for detailed study. The amount of colloid found varied from 20 to 65 per cent in the mineral soils.

2. The colloidal material was studied as to composition of Al_2O_3 , Fe_2O_3 , CaO, organic matter, pH, and exchangeable calcium, and compared with the whole soil.

3. Similar studies were made of each horizon of each of the other soils for comparison of physical properties with chemical composition.

4. All the soils behave similarly to the plastic or non-friable group used by Bennett (2) rather than to the friable or lateritic group. There is some slight lateritic tendency in the Aiken and Olympic series, however.

5. Both the separated colloids and the soils themselves show a high retentive capacity for soluble phosphates. The colloids show a higher retentive capacity than the whole soil.

6. There is not sufficient exchangeable calcium or other bases to account for the entire amount of phosphates retained by either the soils or the colloids.

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THE FERMENTATION OF GLUCOSE AND XYLOSE BY THE NODULE BACTERIA FROM ALFALFA, CLOVER, PEA, AND SOYBEAN

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One of the recognized characteristics of the root nodule bacteria of the *Leguminosae* is the ability to ferment sugars. Although there are many reports concerning the change in reaction of a carbohydrate culture medium brought about by the growth of nodule bacteria, the quantitative data relating to this subject are meagre. Chronologically the first report is that of Mazé (5) in 1898, made in connection with his studies of nitrogen fixation by the organisms in artificial culture. In a medium containing approximately 2 per cent sucrose he found 59 to 68 per cent destruction of sugar in 19 to 22 days. In 1911-12 Fred (3) reported a limited fermentation of maltose and sucrose by various strains of the nodule bacteria; in no case was more than 20 per cent of the sugar utilized in one month's time. In a brief report on nitrogen-fixation by non-symbiotic organisms, Hutchinson (4) in 1922-23 presented a graph of glucose destruction by root nodule bacteria. The strain of this culture was not given. The curve indicates a rapid utilization of sugar in the first 10 days, the concentration falling from 2 per cent to less than 1 per cent during that time. Thereafter there is a gradual decrease in residual sugar until at 60 days approximately 0.4 per cent is left. More extensive tests were reported in 1928 by Anderson, Peterson, and Fred (1). These authors studied the rate of fermentation of glucose by two strains of root nodule bacteria of alfalfa in large cultures. They found that 0.435 gm. per 100 cc. were destroyed in 45 days by one strain and 0.6 gm. per 100 cc. in 41 days by a second strain.

The present work was undertaken to measure the rate at which a typical hexose, glucose, and a typical pentose, xylose, are destroyed by various species of root nodule bacteria and also to determine the total numbers of bacteria at various intervals.

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EXPERIMENTAL METHODS

The general procedure was to grow the organism in a medium consisting of dipotassium phosphate 0.5 gm., magnesium sulfate 0.2 gm., sodium chloride 0.2 gm., calcium sulfate 0.1 gm., carbohydrate 10 gm., yeast water (infusion) 100 cc., and distilled water, 900 cc. In certain experiments either 3 gm. of calcium carbonate or 3 gm. of basic slag (Thomas phosphate meal), was added. Basic slag was chosen because of its use in the fertilizer industry. The reaction was adjusted to a pH of 7.2-7.3. The media were sterilized at 120°C. for 20 minutes on two successive days before inoculation. Sugar determinations were made by the micro method of Shaffer and Hartmann as modified by Stiles, Peterson, and Fred (6). Before samples for quantitative analyses were drawn, purity tests on litmus milk and potato slopes were made.

In some cases where the destruction of sugar was very slow, the percentage of the carbohydrate apparently increased with the age of the culture. It was thought that the apparent increase was due to evaporation of the cultures. To test this assumption the flasks were weighed each time before and after the

TABLE 1

Effect of evaporation on the concentration of glucose in shallow layers of fermenting culture solutions

| TIME | VOLUME | REMOVED FOR ANALYSIS | LOST BY EVAPORATION | GLUCOSE BY ANALYSIS | GLUCOSE CORRECTED FOR EVAPORATION |
|------|--------|----------------------|---------------------|---------------------|-----------------------------------|
| days | cc. | cc. | cc. per day | gm. per 100 cc. | gm. per 100 cc. |
| 0 | 89.0 | | | 1.37 | 1.37 |
| 12 | 84.4 | 10.9 | 0.38 | 1.13 | 1.07 |
| 19 | 70.4 | 9.0 | 0.45 | 1.00 | 0.91 |
| 33 | 55.3 | 9.9 | 0.35 | 0.85 | 0.69 |

removal of the sample for analysis. From the data in table 1, column 4, it is seen that the loss of weight ranged from 0.35 to 0.45 cc. per day and averaged 0.381 cc. for 33 days. The total loss for the whole fermentation period of 33 days amounts to 12.5 cc. Obviously a correction for this amount of evaporation must be made for the concentration of the sugar. The percentages of sugar found by analysis (table 1, column 5) have been corrected for evaporation and the corrected figures are given in column 6. A comparison of these two columns shows that the apparent rate of glucose destruction is considerably slower than the actual rate.

All of the figures obtained by analysis were therefore corrected to the volume after inoculation and then expressed in terms of 100 cc. of culture.

The following pure cultures of root nodule bacteria were used:²

1. *Rhizobium meliloti*—Alfalfa (*Medicago sativa*)
2. *Rhizobium trifolii*—red clover (*Trifolium pratense*)
3. *Rhizobium leguminosarum*—pea (*Pisum sativum*)
4. *Rhizobium japonicum*—soybean (*Soja max*)

² The nomenclature is that proposed by Baldwin and Fred (2).

FERMENTATION OF GLUCOSE

In the preliminary experiment a few sugar determinations were made at intervals upon two cultures inoculated with *Rhizobium meliloti* No. 100. One culture contained calcium carbonate; the other did not. In both cases there was a gradual decrease in the amount of sugar present; about 10 per cent

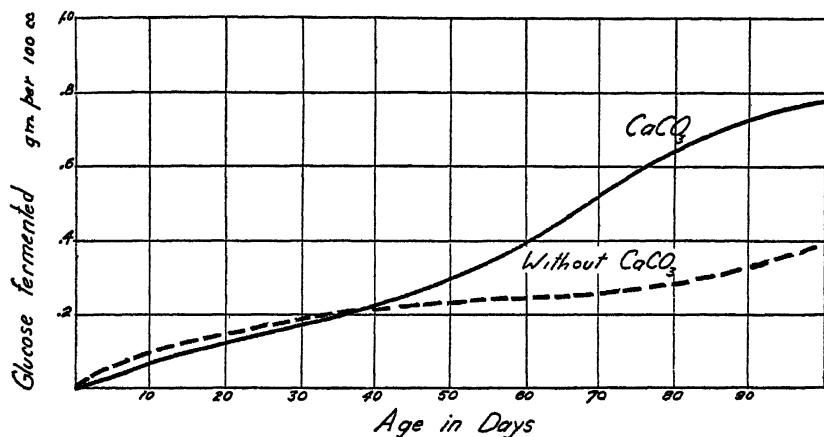


FIG. 1. THE FERMENTATION OF GLUCOSE BY RHIZOBIUM TRIFOLIUM IN A YEAST WATER MEDIUM

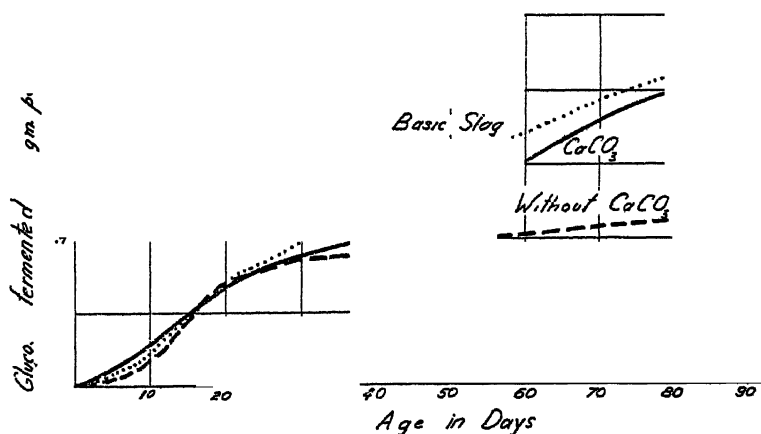


FIG. 2. THE FERMENTATION OF GLUCOSE BY RHIZOBIUM LEGUMINOSARUM IN A YEAST WATER MEDIUM

was destroyed in 20 days. In 60 days about 70 per cent of the sugar in the culture containing calcium carbonate was destroyed. After 100 days, about 20 per cent of the sugar in the culture without calcium carbonate was consumed and 90 per cent of the sugar in the culture with calcium carbonate. These results show that calcium carbonate is beneficial to the growth of the nodule organism.

In the first experiment 200 cc. of the medium in 750-cc. Erlenmeyer flasks were used and seeded with *Rhizobium trifolii* No. 205. The results of this experiment are shown graphically in figure 1.

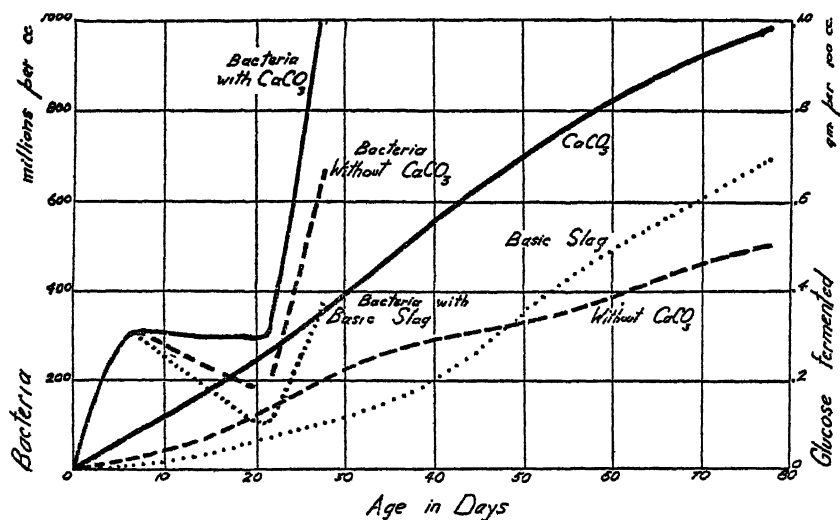


FIG. 3. THE FERMENTATION OF GLUCOSE BY RHIZOBIUM JAPONICUM IN A YEAST WATER MEDIUM

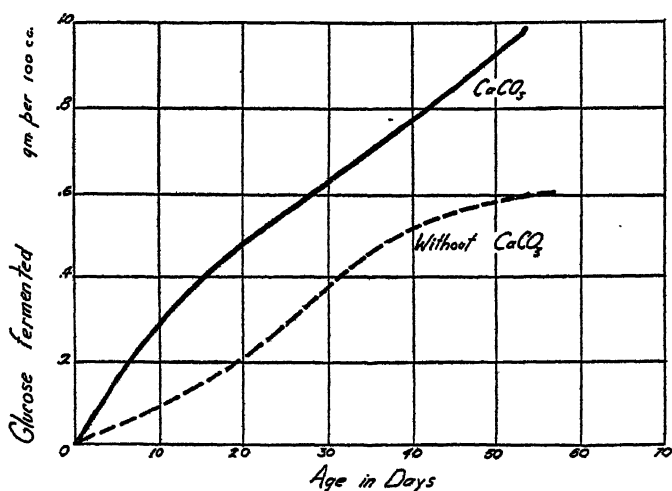


FIG. 4. THE FERMENTATION OF GLUCOSE BY RHIZOBIUM MELILOTI IN A YEAST WATER MEDIUM

In experiment 2, the medium was made up without the glucose and the percentage of the salts doubled. The sugar was then made in a separate water solution and, after autoclaving, 50 cc. of each solution were pipetted into

750-cc. Erlenmeyer flasks. The cultures were inoculated with *Rhizobium leguminosarum* No. 308, *Rhizobium japonicum* No. 504, and *Rhizobium meliloti* No. 100. The data are given in figures 2, 3, and 4.

The following conclusions may be drawn from these data:

The fermentation of glucose is a slow but continuous process without any very marked difference between the cultures studied. The representative of the alfalfa group is perhaps the most active, whereas that of the clover group is the weakest in relation to the sugar destroyed.

If the rate of glucose fermentation of the various cultures be compared it will be seen that the clover and pea bacteria are much alike. The alfalfa and soybean bacteria behave somewhat differently and fail to show any marked decrease in fermentation with an increase in age of culture.

A neutralizing agent practically doubles the quantity of sugar destroyed. Without CaCO_3 , from 40 to 50 per cent of the glucose was destroyed; and with CaCO_3 , the figures are from 70 to 90 per cent.

In the early stages of the fermentation the rate is approximately as rapid in the absence as in the presence of CaCO_3 . Presumably an accumulation of acid produces such a high hydrogen-ion concentration in the absence of CaCO_3 that the continued destruction of sugar is checked. Basic slag shows about the same effect as CaCO_3 . Apparently its action is due to its neutralizing power and not to its phosphorus content.

Because of the limited number of determinations and the difficulty in securing satisfactory counts of the numbers of bacteria, conclusions can be drawn only with considerable reservation. The maximum number was reached in about 7 days, but in some cases a second high point was observed after 28 days.

FERMENTATION OF XYLOSE

The data dealing with the destruction of this sugar are given graphically in figures 5, 6, 7, and 8.

If the same length of fermentation period is chosen, there is no appreciable difference in the degree of fermentation of glucose and xylose. Likewise, there is no particular difference in the rate at the time of maximum fermentation. After the maximum rate is reached the fermentation of xylose shows the same gradual decrease with an increase in the age of the culture that was observed with glucose. Apparently the pentose sugar is just as good a source of carbon as the more common hexose.

Although the alfalfa organism is the most active in the fermentation of glucose, it is the slowest fermenter of xylose. The other three organisms ferment the pentose at about the same rate.

As was noted with glucose, a neutralizing agent increases the percentage of sugar destroyed. That this is entirely a question of reaction is shown by the rate of sugar destruction in the early stages of the fermentation; up to 10 days, approximately the same quantity of sugar is destroyed in the presence as in

the absence of CaCO_3 . Although there are minor variations, they have no particular significance.

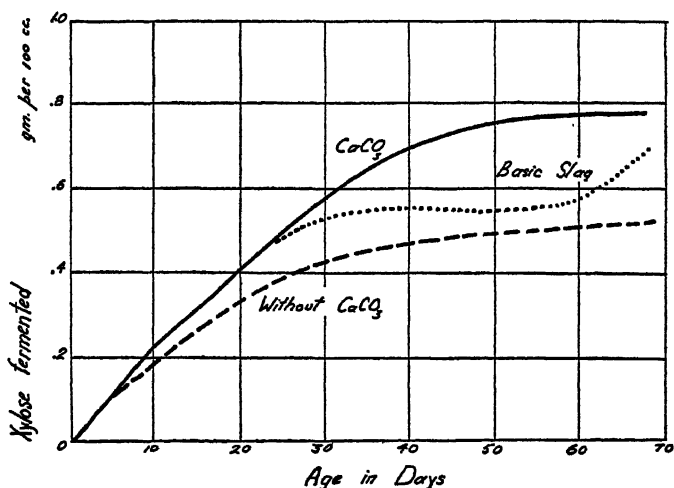


FIG. 5. THE FERMENTATION OF XYLOSE BY RHIZOBIUM TRIFOLIUM IN A YEAST WATER MEDIUM

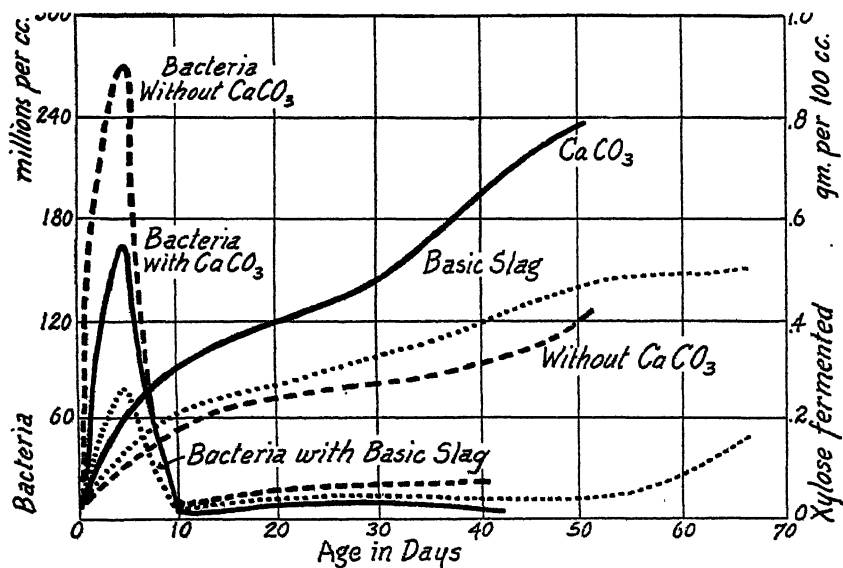


FIG. 6. THE FERMENTATION OF XYLOSE BY RHIZOBIUM LEGUMINOSARUM IN A YEAST WATER MEDIUM

H-ion determinations on the unneutralized cultures at the end of the fermentation give further evidence to explain the slow fermentation. Without CaCO_3 or basic slag in a xylose medium, pH values for the different microorganisms

were found as follows: *Rhizobium meliloti*, 5.6; *Rhizobium trifolii*, 5.0; *Rhizobium japonicum*, 4.6. The pH values in a glucose medium were: *Rhizobium meliloti*, 5.2; *Rhizobium trifolii*, 5.4; and the *Rhizobium leguminosarum*, 5.6.

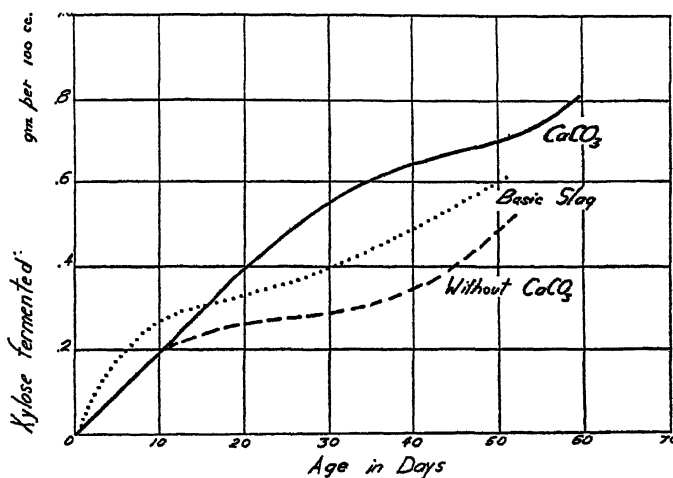


FIG. 7. THE FERMENTATION OF XYLOSE BY *RHIZOBIUM japonicum* IN A YEAST MEDIUM

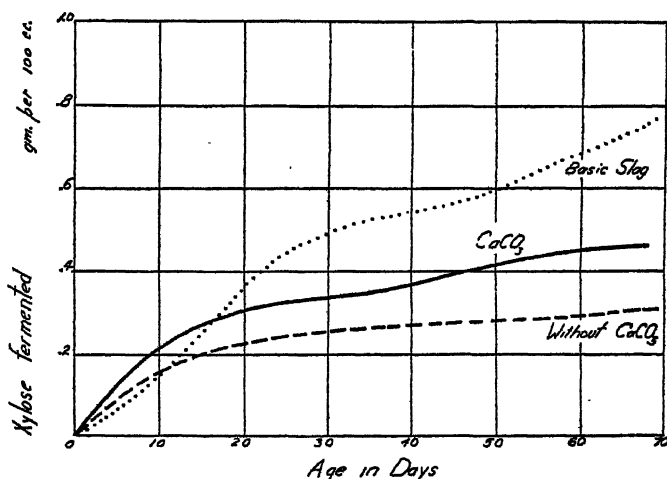


FIG. 8. THE FERMENTATION OF XYLOSE BY *RHIZOBIUM meliloti* IN A YEAST WATER MEDIUM

As was observed in the fermentation of glucose, a maximum in the numbers of bacteria was reached in about seven days. In many cases a second maximum point was observed, but at a slightly later date than with glucose. The variation in the numbers of bacteria may be explained in part by the frequently observed formation of a thick gum which makes it difficult to break up the clumps of organisms.

SUMMARY

1. Laboratory experiments on the rate of sugar destruction by four species of nodule bacteria, *Rhizobium meliloti*, *Rhizobium trifolii*, *Rhizobium leguminosarum*, and *Rhizobium japonicum* in yeast water carbohydrate media were conducted. A typical hexose, glucose, and a typical pentose, xylose, were used.

2. Cultures of *Rhizobium meliloti*, *Rhizobium trifolii*, *Rhizobium leguminosarum*, and *Rhizobium japonicum*, when grown in a sugar medium with calcium carbonate or basic slag present, show a greater destruction of sugar than in a similar medium without these basic substances. In the presence of these substances approximately three-fourths of the sugar was destroyed in 75-day-old cultures, whereas in a medium without these substances only one-half of the sugar was destroyed in the same time.

3. The rate of fermentation of glucose and xylose each day is usually faster in the young cultures, showing a gradual decrease with an increase in age.

4. As a rule the maximum number of bacteria is reached within 10 days. In some cases there were several successive increases and decreases in numbers.

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- (2) BALDWIN, I. L., AND FRED, E. B. 1929 Suggestions concerning the nomenclature of the root nodule bacteria of the Leguminosae. *Jour. Bact.* 17: 141-150.
- (3) FRED, E. B. 1912 A physiological study of the legume bacteria. *Va. Agr. Exp. Sta. Ann. Rpt.* 1911-12: 145-173.
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- (5) MAZÉ, M. 1898 Les microbes des nodosités des Légumineuses. *Ann. Inst. Pasteur*, 12: 1-25; 128-155.
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BOOK REVIEWS

REVIEWS OF AND REFERENCES TO BOOKS OF INTEREST TO THE READERS OF SOIL SCIENCE

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New Jersey Agricultural Experiment Station

Received for publication July 24, 1929

Dictionary of Bacteriological Equivalents. By WILLIAM PARTRIDGE. Baillière, Tindall, and Cox, London; and Williams & Wilkins Company, Baltimore, 1927. Pp. xi + 141.

A very convenient reference book for microbiologists, biochemists, and biologists. The equivalents given are French-English, German-English, Italian-English, and Spanish-English.

Lectures on Plant Pathology and Physiology in Relation to Man. W. B. Saunders Company, Philadelphia and London, 1926-27. Pp. 207, figs. 16.

This contains (a) a group of lectures on plant pathology and physiology in relation to man, given during the autumn and winter of 1926-27 under the auspices of The Mayo Foundation and the local chapter of Sigma Xi at Rochester, Minn.; the Medical School of the University of Wisconsin, Madison, Wis.; the Graduate School of the University of Minnesota, Minneapolis, Minn.; the Graduate School of the University of Iowa, Iowa City, Iowa; the Iowa State College, Ames, Iowa; and the Des Moines Academy of Medicine, Des Moines, Iowa. The authors of these lectures were Louis Otto Kunkel, Henry Chandler Cowles, George Herbert Coons, Elvin C. Stakman, Herbert Hice Whetzel, and Winthrop John Vanleuven Osterhout.

Soil Characteristics. By PAUL EMERSON. McGraw-Hill Book Company, New York, 1925. Pp. x + 222, pls. 1, figs. 5.

The book deals with physical, chemical, and microbiological methods involved in the study of soils.

Handbook of Fertilizers. By A. F. GUSTAFSON. Orange Judd Publishing Company, Inc., New York; and Kegan Paul, Trench, Trübner & Co., Ltd., London, 1928. Pp. 122, figs. 18.

As stated by the author, "This little book is an attempt to supply accurate, up-to-date information as to the source and make-up of commercial fertilizers. Special stress is laid on the effects of fertilizers on soils and crops in the hope of aiding the user to make a wise choice for his individual soil conditions."

The Economics of Land Reclamation in the United States. By RAY P. TEELE. A. W. Shaw Company, Chicago and New York; and A. W. Shaw and Company, Ltd., London, 1927. Pp. xv + 337.

In this book is presented a discussion of the experience of the United States in the reclamation of land for agricultural use, with occasional reference to the experience of other countries in this field. The subject is discussed from the economic standpoint rather than from the engineering or the cropping standpoint, although it is necessary to consider both engineering and crop production, since crop production is the object of land reclamation and engineering is the means of accomplishing this objective.

Phosphoric Acid, Phosphates, and Phosphatic Fertilizers. By WM. H. WAGGAMAN assisted by HENRY W. EASTERWOOD. The Chemical Catalog Company, Inc., New York, 1927. Pp. 370, figs. 58.

The book contains a mass of valuable information and is well supplied with references.

Foundations of Silviculture upon an Ecological Basis, volume I. By JAMES W. TOUMEY. John Wiley & Sons, Inc., New York; and Chapman & Hall, Ltd., London, 1928. Pp. xxv + 438, figs. 11.

The author discusses in chapters I to X the site factors, and in chapters XI to XV the forest. The book is well written and should be given a place on the shelves of every reader interested in soils and their products.

The Practice of Silviculture. By RALPH C. HAWLEY. John Wiley & Sons, Inc., New York; and Chapman & Hall, Ltd., London, 1929. Second edition, pp. xiii + 335, figs. 69.

The book deals with applied silviculture. It is well illustrated and contains an appendix of forest terminology.

Minerals in Pastures and Their Relation to Animal Nutrition. By J. B. ORR assisted by HELEN SCHERBATOFF. H. K. Lewis & Co., Ltd., London, 1929. Pp. x + 150, figs. 2.

Within recent years much new information has become available on the composition of forage crops and of pasture grasses in particular. Much of this new information is summarized in this book.

Handbook of Microscopical Technique. Edited by C. E. MCCLUNG. Paul B. Hoeber, Inc., New York, 1929. Pp. xiv + 495, figs. 43.

A number of leading authorities have collaborated in creating a valuable treatise. As noted in the preface, "In two previous texts, *General Cytology* and *Special Cytology*, edited by Dr. Cowdry, experts in various fields of biology have presented the results of their investigations. In the present work, which extends the series, the methods involved in these and similar studies are given."

Chemistry in Medicine. Edited by Julius Stieglitz. The Chemical Foundation, Inc., New York. Pp. xxi + 757, pls. 9, figs. 15, chart 1.

The Chemical Foundation is to be felicitated on having got out this book and on having made the information contained in it widely available.

Field-Crop Enterprises, Including Soil Management. By KARY C. DAVIS. J. B. Lippincott Company, Philadelphia, London, Chicago, 1928. Pp. vi + 528, figs. 260.

We are informed by the author that the book is intended primarily as a text in vocational schools. At the same time, it should also prove helpful to progressive farmers, since it offers in a compact and popular way information of wide interest.

Scientific Preservation of Food. By THOMAS M. RECTOR. John Wiley & Sons, Inc., New York; and Chapman & Hall, Ltd., London, 1925. Pp. xi + 213.

There is much in this book that will interest microbiologists. The author points out in the preface that: "For the past 12 years the writer has been engaged in applying the principles of chemistry and bacteriology to the problems of the food manufacturing industry. This experience has included extensive analytical work, solution of spoilage problems, and finally the creation of new products and processes."

Botany. By WILLIAM J. ROBBINS AND HAROLD W. RICKETT. D. Van Nostrand Company, Inc., New York, 1929. Pp. xxiii + 535, figs. 384.

The intimate relations that exist between plant and soil science will justify the student of soils in reading this book carefully.

Ackerbaulehre, volume 2. Edited by TH. ROEMER. Paul Parey, Berlin, 1929. Pp. xvi + 564, colored pls. 4, figs. 130.

This work represents an ambitious effort on the part of the editor and his associates to cover much of the field of soil science. Part I deals with the origin, properties, and classification of soils; part II, with the biology of soils; part III, climate in its agricultural relations; part IV, reclamation methods; part V, tillage; part VI, seed, germination, and quality of seed; part VII, feeding and fertilization of plants; part VIII, the control of weeds; part IX, catch crops and green manures; part X, diseases of plants; and part XI. crops and their storing and conservation.

Die Untersuchung und Begutachtung von Düngemitteln, Futtermitteln, Saatwaren und Bodenproben. By PAUL KIRSCH assisted by ALBERT KABITZSCH. Paul Parey, Berlin. Second edition, pp. xxii + 386.

The book is devoted largely to a consideration of analytical methods employed in the German agricultural experiment stations for ascertaining the composition of fertilizers, feeding stuffs, and soils. Methods are also described for determining the quality of agricultural seeds.

Handbuch der Bodenlehre, volume I. Edited by E. BLANCK. Julius Springer, Berlin, 1929. Pp. viii + 335, figs. 29.

As is indicated on the title page, the book deals with the principles involved in the genesis of soils. The editors tell us in the preface that, in dealing with soil science, account must be taken of other sciences, such as botany, tillage, and climatology. An attempt has been made by the collaborators to outline the broad limits of the field. The discussion follows in logical sequence, and should interest not only the student of soils, but also all progressive landowners.

RELATION OF CALCIUM TO THE NODULATION OF SOYBEANS ON ACID AND NEUTRAL SOILS

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As a result of recent tests on the effects of calcium-bearing soil treatments, such as lime, acid phosphate, and calcium salts, upon nodulation of soybeans, there is an indication that the beneficial effect of these materials is essentially one of calcium stimulation. Frequent and repeated reports of failures to obtain nodulation of legumes by the pure culture method on certain acid soil types of northeastern Missouri and southeastern Illinois led to a search for the responsible factors. Soybeans, as an acid tolerant legume, have taken a prominent place in this territory within recent years, but thorough inoculation of even this crop has been difficult unless the soil was well limed. A study of the beneficial effects of lime on soybean inoculation, reported herein, leads to the belief that much of this is due to the element calcium.

HISTORICAL

Harper and Murphy (7), have recently given a review of the literature dealing with the factors affecting nodule formation by soybean plants. Other recent papers summarize the literature similarly, including specific phases of inoculation. Wilson (14), Scanlan (12), and Karraker (9), have each reported an increase in nodulation in consequence of the application of calcium as the carbonate or as a soluble salt. Alway (1), who was comparing the effectiveness of inoculation of alfalfa by soil transfer with that by pure cultures on lime-deficient sandy soils, found these two methods of equal efficacy when the land had been limed well in advance of seeding. But when the land had not been limed, the soil transfer method was far more effective. An increase in the amount of culture, many times beyond the usual rate, did not make it as effective as soil transfer. This seems to indicate an adaptation of the organism to a lime-deficient soil habitat in consequence of several previously grown, host crops. Bryan (4), in a study of the effect of acid soil reactions on nodulation of soybeans, found that, in general, the hydrogen-ion relations for the organisms tend to be the same as those for the host plant. He secured a

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maximum nodulation at pH 6.5 and none below 4.9, although the critical hydrogen-ion concentration for the organisms was found to be pH 3.5 to 3.9. Scanlan (12) concluded that hydrogen-ion concentration must have no direct effect upon inoculation of soybeans by *B. radiculicola*. Although both calcium carbonate and calcium acetate stimulated inoculation tremendously, the carbonate neutralized the hydrogen-ion concentration and the acetate had no effect upon it. Fellers (6) noted that the bacterial infection of roots did not take place readily on acid soils even when a good supply of bacteria was present.

Karraker (9), working with alfalfa, single plants of which he grew with part of the roots in a limed and part in a lime-deficient, acid soil, found that there was a difference in the nodule formation of the two parts of the root system, and that this difference was as great as the difference between the nodulation of plants grown wholly on the limed and those on the lime-deficient soils. He concluded that the effect of soil reaction upon nodule formation must be one of localized character in the plant, a direct effect of soil hydrogen-ion concentration on the bacteria in the nodules, or an antecedent effect of the soil on the bacteria while they are existing non-symbiotically in the soil.

It has been pointed out by Lohnis and Smith (10) that bacteria undergo a fairly definite cyclic change. Bewley and Hutchinson (2) found similar cyclic changes and that these are even specific with reference to cultural conditions. They stated that as long as there was sufficient available carbohydrate to support growth, the organism remained in the motile rod form with no changes. Their "pre-swarmers" (one of their cyclic forms), could be induced by the addition of calcium or magnesium carbonate to the medium. Of a considerable number of compounds other than carbohydrates, calcium phosphate alone was capable of bringing about the change from "pre-swarmers" to rods. The response to the reaction of the soil, in the main, was a rapid change from the normal rod form to "pre-swarmers" in calcareous soils; a production of a highly vacuolated form and the eventual death of the organisms in acid soil; and a continuous growth without significant change of form in a slightly alkaline soil. Thornton and Gangulee (13), in an even more recent investigation along this line, also noted a similar, regular cycle of change, in which unbanded rods, cocci, and banded rods successively followed each other. They stated that an increase in the percentage of cocci was associated with increased bacterial numbers and with the appearance of motile forms. They found that by modifying the liquid used to suspend the inoculum added to the soil, the time of appearance of cocci in predominance could be altered, and on this basis they recommend the inoculation of the soil with a bacterial suspension in milk to which is added 0.1 per cent of calcium acid phosphate.

That calcium may play some significant part in establishing legume bacteria in certain lime-deficient soils was suggested by previous workers and served as the main hypothesis in the work reported herein.

EXPERIMENTAL

Part I

Increased nodulation of soybeans, upon an already well-inoculated soil, resulted from the use of calcium treatment on such soil in the greenhouse. This was an acid soil (pH 5.5), taken from the experimental field, and already well inhabited with the symbiotic organism in consequence of inoculated crops of soybeans of the three preceding seasons. Limestone, equivalent to 4 tons per 2 million pounds, was added to part of the soil. The seed from a single mother plant was used for a stand of 5 plants per pot on 30 pots from both the limed and the unlimed soil. They were grown for 5 weeks, after which the uniform and healthy plants were taken up and the nodules counted. Although Erdman (5), has presented an argument for the importance of the size as well as the number of nodules in determining the effectiveness of inoculation, the numbers only were taken. The data are summarized in the first half of table 1.

The results show an increase of 336 per cent in numbers of nodules formed as a result of liming, even though the soil was already well inoculated with the organism. In spite of the fact that these data show a correlation between inoculation and neutralization of the soil acidity, this does not necessarily establish a causal relationship.

Part II

In consequence of the fact that an application of calcium as the carbonate, produced an important increase of nodulation on an already well-inoculated soil, and of the belief that this stimulation was not necessarily the result of a change in hydrogen-ion concentration, or at least not entirely so, a test was made of the effect of calcium, as the chloride, upon the inoculation of soybeans by the pure culture method on an acid soil which was sterile with regard to *B. radicicola* of soybeans. To each of 30 pots of a rather heavy Union silt loam (pH 5.4), there were added at planting time 25 cc. of a solution of calcium chloride supplying calcium equivalent to that of 200 pounds of calcium carbonate per 2 million of soil. Thirty pots of the untreated soil were planted also. Liberal quantities of an inoculating suspension were supplied directly to the beans at the time of planting. Eight pots of untreated and uninoculated soil were planted as checks. When, at the end of 5 weeks, the examination for nodules revealed none formed, 16 pots each of the inoculated, calcium treated and of the inoculated, untreated soil were immediately replanted with sprouted beans in the same pots. After 5 weeks of growth, these were likewise carefully taken up, washed, and examined for nodules. The complete data are summarized in the latter half of table 1.

The nodulation of the beans of the second planting indicates that the calcium as a chloride has a stimulating effect upon the longevity and viability of the organism in the acid soil. This agrees with Scanlan's results of increased viability of the organism in water cultures.

Part III

Since the addition of a small amount of a neutral calcium salt to an acid soil (pH 5.4) had kept *B. Radicicola* viable within the soil from the time it was applied by pure cultures on the first crop until the second planting, and since an addition of large amounts of calcium carbonate produced important in-

TABLE 1
Nodulation of soybeans on acid soils as influenced by calcium compounds

| SOIL TREATMENT | NUMBER OF POTS | TOTAL NUMBER OF PLANTS | RANGE IN NODULES PER POT | AVERAGE NODULES PER PLANT | PER CENT INCREASE |
|---|-----------------------|------------------------|--------------------------|---------------------------|-------------------|
| <i>Soil already inoculated given calcium carbonate</i> | | | | | |
| None..... | 30 | 130 | 10-81* | 12.0 | |
| Calcium carbonate—4 tons..... | 30 | 133 | 64-247† | 40.2 | 336 |
| <i>Sterile soil given calcium chloride with inoculation</i> | | | | | |
| First crop { | None..... | 30 | 130 | — | — |
| | Calcium chloride..... | 30 | 133 | — | —‡ |
| Second crop { | None..... | 16 | 72 | — | —‡ |
| | Calcium chloride..... | 16 | 80 | 1-26 | 1.8 |

* Three pots exceeded this range greatly, having 110, 140, and 142 nodules.

† Two pots exceeded this range greatly, having 297 and 380 nodules.

‡ Three plants had one nodule each in these trials.

TABLE 2
Nodulation of soybeans grown with part of root system in calcium-treated soil and part in untreated soil

| TREATMENT | PLANTS WITH DIVIDED ROOTS | | | CHECK PLANTS | | | |
|--|---------------------------|-------------------------------|-------------------------|----------------------|-------------------|------------------|-------------------|
| | Number of plants | Nodule production | | Calcium-treated soil | | Untreated soil | |
| | | Roots in calcium-treated soil | Roots in untreated soil | Number of plants | Number of nodules | Number of plants | Number of nodules |
| Uninoculated..... | 4 | 0 | 0 | 6 | 0 | 5 | 0 |
| Inoculated..... | 23 | 160 | 77 | 28 | 494 | 31 | 302 |
| Average per plant..... | | 6.95 | 3.34 | | 17.64 | | 9.74 |
| Per cent increase through calcium..... | | 208 | | | 181 | | |

creases in nodulation of soybeans on an already well-inoculated soil, it was thought possible to determine whether this stimulating effect was due, (a) to calcium within the plant, (b) to an effect of calcium upon the organism in the soil, or (c) to an effect of calcium upon the soil as the habitat of the organisms, by growing plants so arranged that one part of the root system

of each was growing in an acid soil and the other part in the calcium-treated soil.

Soybean seedlings were grown in sterile sand from 10 to 14 days, or until the lateral roots about an inch long were sufficient to support the plant. These seedlings were taken up, washed, and the tap roots cut off just below the longest lateral roots. They were then planted with half of their roots on one side and half on the other side of the water-tight partition of a two-compartment pan. The moist acid soil was filled in around the roots on one side, and moist calcium-treated soil on the other. Liberal quantities of the inoculating suspension were supplied directly to the roots. The soil used was a Putnam silt loam (pH 5.14). Five plants were planted with their roots divided by the partition and five more plants with their tap roots similarly pruned were planted wholly on each side of the partition as checks. Although there was a high mortality of plants, those that lived grew satisfactorily for the five weeks, after which a count of the nodules was made. The results are given in table 2.

The increase in nodulation on the parts of the root systems growing in the calcium-treated soil over those parts growing in the untreated soil was comparable to the increase obtained in the checks or those whose entire root system was within a single soil treatment. This agrees with the results obtained by Karraker (9) on alfalfa. Although this type of experiment is unsatisfactory because of unequal development of the divided parts of the root, the results indicate that the stimulating effect of the calcium upon nodulation was due to an effect upon the bacteria in the soil, or to a physiological effect within the plant. In addition, the effect was local in character, and limited to the roots. The calcium was not translocated to all parts of the plant root system sufficiently to make its influence uniform on the degree of inoculation, at least not within the time limits of this experiment.

Part IV

The preceding results raised the question whether there is an effect upon nodulation by the calcium already within the plant tissues. An attempted answer was undertaken by growing some soybean seedlings in calcium-free and some in calcium-bearing substrates and then transplanting from both into an inoculated soil. The calcium-free substrate was prepared by treating sand with 5*N* hydrochloric acid for 3 hours, washing with water until acid-free according to the silver nitrate test, drying, and sterilizing in an oven at 110°C. for 48 hours. For the calcium-bearing substrate, calcium carbonate was mixed with the same quartz sand at the rate of 10,000 pounds per 2 million. This was also sterilized in the oven 110°.

The total yield of beans from a single plant was sterilized, germinated for 24 hours, and planted into pots of these sterile substrates, half into the calcium-free and half into the calcium-bearing sand. The pots were set to their shoulders into moist soil, which was sterile with regard to *B. radiculicola*, and after 10 or 11 days the plants were taken up, washed, and replanted to the

inoculated soil which had been prepared and sifted at a suitable moisture content into ordinary greenhouse flats. Both the calcium-bearing and calcium-starved seedlings were grown simultaneously on their respective halves of the same flat and within the same soil. Seedlings so treated were transplanted and grown on two different soils, one an acid, lime-deficient soil, and the other, a neutral, fertile, garden soil. No inoculation was added, since both soils had grown well-inoculated crops of soybeans during two consecutive seasons just previous to this test. After a growth of 5 weeks, the plants were taken up readily without injury to the roots and the nodules counted. The data from the count are summarized in table 3. Included in the table are also the analytical data giving, (a) the calcium content of the soybean seeds from a single, similar plant, (b) the calcium content of 10-day-old calcium-bearing and calcium-starved seedlings, and (c) the total electro-dialyzable calcium of the soils as determined by Bradfield's (3) method of measuring the total electro-

TABLE 3
Nodulation of soybeans in neutral and acid soils as influenced by the calcium in the seedlings

| SOIL CHARACTER | pH | SEEDLING TREATMENT | NUMBER OF PLANTS | NODULE NUMBERS PER PLANT | | CALCIUM CONTENT | | |
|----------------|-------|--------------------|------------------|--------------------------|---------|-------------------|------------------------------------|---------------|
| | | | | Range | Average | Per 100 seedlings | Electro-dialyzable per 10 gm. soil | Per 100 seeds |
| Neutral..... | 7.8 { | None | 60 | 12-77 | 36.6 | 17.07 | 24.07 | 6.85 |
| | | Calcium | 67 | 9-67 | 38.9 | 30.14 | | |
| Acid..... | 5.5 { | None | 69 | 1-7 | 3.4 | 17.07 | 11.78 | 6.85 |
| | | Calcium | 79 | 2-25 | 15.1 | 30.14 | | |

dialyzable base. These analyses were made in order to correlate the nodulation with the calcium content of the plants as influenced by the treatments.

The increased nodulation of the calcium-bearing seedlings on the calcium-deficient, acid soil demonstrates that the presence of calcium within the plant increases nodulation of soybeans on such soil. On the other hand, the lack of difference in nodulation of the seedlings on the neutral, calcium-laden soil indicates that the presence of this element within the plant on a calcium-sufficient soil does not affect nodulation, or that if it does, the calcium-starved plants are able to take calcium from the soil rapidly enough to offset the measurable differences in nodulation.

The increase in calcium content of the calcium-starved seedlings over that in the seed, as shown by the analytical data, was due to the calcium that was carried back into the acid-extracted sand by the tap water with which it was washed. An elimination of this factor might have served to intensify further the differences obtained.

Part V

Since calcium exerted an intimate effect upon inoculation by the organism *B. radiculicola*, this effect was deemed possible through an inter-relation with the soil colloids, the main chemically reactive part of the soil. It is known that the colloids are highly absorptive, and that minute quantities of calcium are effective in flocculating them, hence this phase of the experiment was undertaken to detect such possible relation.

A suspension in distilled water of the organisms from several agar cultures was added to a 0.4 per cent solution of colloidal clay in a ratio of four parts of the bacterial suspension to five parts colloidal solution. To this mixture was added one part of water containing the desired amount of flocculating agent.

TABLE 4
Nodule numbers on soybeans inoculated by colloidal clay suspensions of bacteria

| KIND OF INOCULATING SUSPENSION | RANGE IN NUMBER OF NODULES PER POT* | DESCRIPTION OF NODULATION |
|-------------------------------------|--|--|
| Distilled water..... | 72-90 | Variable size. Scattered over entire root system |
| Colloidal clay..... | 84-112 | Uniform size. Clumped at plant crown |
| Tap water..... | 56-71 | Variable size. Well scattered |
| No inoculation..... | 0-0 | Plants yellow. Grew for time of test |
| Full inoculation..... | 98-161 | Variable size. Well distributed |
| Calcium chloride supernatant..... | 0-6 | Not over one plant per pot infected |
| Calcium chloride flocculant..... | 64-133 | Variable size. Clumped at plant crown |
| Potassium chloride supernatant..... | 85-169 | Variable size. Well distributed |
| Potassium chloride flocculant..... | 148-162 | Indiscriminate size. Clumped at base, some scattered |

* Duplicate pots were grown with 5 plants each.

Those mixtures left unflocculated received the equivalent of distilled water. Thus the resulting solutions contained 0.2 per cent colloidal clay and equal numbers of organisms throughout. These were made up in units of 100 cc. in test tubes. The chlorides of potassium and calcium were used as comparative flocculating agents at the rate of 0.5 milliequivalents, or the minimum requirement of potassium chloride as electrolyte at this concentration of colloidal clay. Mixtures of the organism at the same concentrations in the natural colloid, in distilled water, and in tap water were set up as checks.

The tubes were incubated for 7 days, after which the liquid supernatant to the flocculated clay, the flocculated clay itself, the natural colloidal clay, and the suspensions in water, were tested for the presence of the viable organism by applying specific quantities to sterile, germinated soybeans as they were

planted into sterile sand. At the end of 5 weeks the plants were taken up and the nodules per plant counted. The data are presented in table 4. Plate 1 shows clearly the type and extent of nodulation of the roots from the various solutions.

The results obtained in this experiment were duplicated almost identically in a repetition of the experiment 6 weeks later. The nodulation obtained indicated that the colloidal clay absorbed the bacteria but did not destroy their viability. Flocculation of the clay with calcium chloride carried the organisms down and retained them within the flocculant. This was not the case for the potassium chloride. In the potassium chloride the supernatant was as effective for inoculation as the flocculant.

In order to verify the accuracy of this test and to determine whether the calcium chloride or the colloid is the active factor in carrying the organisms out of suspension, this experiment was repeated. Platings were made from the

TABLE 5
Plate counts of B. radicicola as influenced by colloidal clay treatments

| PORTION OF TREATMENT SAMPLED | AVERAGE COUNT PER CUBIC CENTIMETER |
|--|------------------------------------|
| Supernatant to calcium flocculant..... | 8 |
| Supernatant to potassium flocculant..... | 13,100 |
| Upper half calcium-bacteria suspension..... | 200 |
| Upper half potassium-bacteria suspension..... | 7,000 |
| Lower half calcium-bacteria suspension..... | 4,300 |
| Lower half potassium-bacteria suspension..... | 1,000 |
| Supernatant to centrifuged inoculated colloid..... | 22,100 |
| Inoculated colloid—not centrifuged..... | 9,550,000 |
| Tap water suspension..... | 3,600 |

solutions into sterile petri dishes at the time of planting. Also, the pure bacterial suspensions were flocculated by potassium chloride and calcium chloride and then plated. A sterile colloidal clay inoculated 7 days previously was also plated. The effectiveness of the absorption of the bacteria by the colloid was tested by centrifuging the colloidal material out of an inoculated colloidal clay and then plating the centrifuged solution. The counts are given in table 5.

The relation of the calcium to the retention of the bacteria by the flocculated clay, as previously found, was substantiated in this trial. The liquid, supernatant to the potassium chloride flocculant contained over 13,000, whereas that over the calcium chloride flocculant contained but 8 bacteria per cubic centimeter, showing that the calcium flocculated clay carried the bacteria out almost completely whereas the potassium flocculated clay did not. Calcium used independently of the clay, carried out the bacteria, since the water suspension given potassium chloride contained a count of 7000, whereas the treatment with calcium chloride reduced this to 200 per cubic centimeter. In

comparing the inoculated colloid suspension with the same after centrifuging, the number of about ten million in the former was reduced to about 22,000 in the supernatant in consequence of centrifuging.

These data suggest that though the clay carries the bacteria out of suspension, certainly the calcium does likewise, whether used alone or whether combined with the clay colloid. When the calcium is used in conjunction with the clay, however, a more nearly complete removal of the organisms is obtained. This is no doubt due to the simultaneous coagulation of the bacteria and to the flocculation of the colloid. This does not hold true for the potassium chloride.

TABLE 6
Nodulation of soybeans on acid soils in field treatments of calcium

| INOCULATION TREATMENT | MARION SILT LOAM* | | | | PUTNAM SILT LOAM (BETTER PHASE)† | | | |
|------------------------------------|-------------------|--------------------------|-----------------------------|-------------------------|----------------------------------|--------------------------|-----------------------------|-------------------------|
| | Nodules per plant | Per cent infected plants | Nodule | | Nodules per plant | Per cent infected plants | Nodule | |
| | | | cc. Volume, 1000 per nodule | Weight, mgm. per nodule | pH | | cc. Volume, 1000 per nodule | Weight, mgm. per nodule |
| None‡..... | 0.2 | 20.0 | | | 6.4 | 0 | 0 | |
| Culture..... | 0.6 | 6.6 | 900.0 | 133.0 | 6.1 | 3.5 | 43 | 9.5 |
| Culture and calcium chloride..... | 26.6 | 100.0 | 24.1 | 24.4 | 6.0 | 3.3 | 67 | 25.0 |
| Culture and calcium nitrate..... | 22.9 | 100.0 | 28.6 | 28.9 | 6.0 | 3.6 | 50 | 23.3 |
| Culture and calcium hydroxide..... | 22.9 | 100.0 | 21.1 | 23.0 | 6.0 | 8.9 | 83 | 33.4 |
| Inoculated soil..... | 16.0 | 100.0 | 31.2 | 30.0 | 6.4 | 2.6 | 57 | 40.2 |
| Soil and calcium chloride§..... | 13.4 | 100.0 | 45.5 | 44.4 | 6.4 | 6.6 | 87 | 19.7 |
| Soil and calcium nitrate¶..... | 11.6 | 100.0 | 53.5 | 58.3 | 6.4 | 3.7 | 60 | 25.4 |

* The initial soil contained 0.9785 as total base (cc. normal acid) and 23.3 mgm. as electro-dialyzable calcium per 10 gm. soil.

† The initial soil contained 0.7563 as total base (cc. normal acid) and 18.2 mgm. as electro-dialyzable calcium per 10 gm. soil and had a pH of 5.6.

‡ About 30 plants were examined in each case. They contained 1.23 per cent nitrogen in the tops and 0.76 per cent in the roots.

§ The plants in this treatment contained 2.39 per cent N in the tops, 2.04 per cent in the roots.

¶ The plants in this treatment contained 2.16 per cent N in the tops, 2.13 per cent in the roots.

Part VI.—Field Trials

After finding that applications of lime may stimulate nodulation on an acid soil already inhabited by the organisms, and that small amounts of calcium in the soil, as well as small amounts within the plant tissues, are important in stimulating nodulation, it seemed quite plausible that liming a soil may exert its influence not wholly as a secondary effect through the correlation of hydrogen-ion concentration, but also in consequence of its content in calcium. Work was done in the field to test whether small amounts of soluble calcium

The work was done in cooperation with farmers experiencing difficulty in getting inoculation on unplowed land. The soybeans were inoculated at planting time with a tested strain of the organism, and applications of calcium chloride, calcium nitrate, calcium acid phosphate, and calcium hydroxide were made through fertilizer attachments on the seeding machinery. In addition to these, tests were made using inoculated soil, both with and without calcium salt treatments. The salts, including calcium chloride and calcium nitrate, were

| INOCULATION TREATMENTS | PUTNAM SILT LOAM (ROLLING PHASE)* | | | | PUTNAM SILT LOAM (FLAT PHASE)† | | | |
|--|-----------------------------------|--------------------------|---------------------|--------------|--------------------------------|--------------------------|---------------------|--------------|
| | Nodules per plant | Per cent plants infected | Average nodule | | Nodules per plant | Per cent plants infected | Average nodule | |
| | | | Volume, cc. 1000 | Weight, mgm. | | | Volume, cc. 1000 | Weight, mgm. |
| None (a) | 0.6 | 20 | 105.5 | 106.7 | 0 | 0 | | |
| Culture | 10.2 | 100 | 48.9 | 51.1 | 5.6 | 66 | 30.1 | 30.1 |
| Culture and calcium chloride (b) | 5.2 | 90 | 31.2 | 31.8 | Dialyzable | | | |
| Culture and calcium nitrate | 9.2 | 93 | 29.3 | 29.9 | Base | | Calcium | |
| Culture and acid phosphate | 8.0 | 100 | 37.5 | 38.3 | (a) | 0.7665 | | 14.5 |
| Inoculated soil | | | | | (b) | 0.9647 | | 20.6 |
| Soil and calcium chloride | Dialyzable | | | | 3.8 | 83 | 47.8 | 50.4 |
| Soil and calcium nitrate | | | | | 2.1 | 57.1 | 50.9 | 52.1 |
| Soil and limestone† ... | | | | | 1.1 | 33 | 26.4 | 28.2 |
| | 25 | 100 | 26.8 | 27.7 | | | | |

‡ Limestone applied was equivalent to 5 tons per 2,000,000 pounds soil.

The data show that the culture inoculation was successful on the Marion Silt Loam in every case where it was supplemented by applications of small amounts of calcium salts, but failed wherever the calcium was omitted.

These differences were very noticeable in the color of the plants. The significant differences in the crop and inoculation on this soil in consequence of the calcium treatment are shown in plate 2. Soil inoculation was successful on this soil type without added calcium. The inclusion of calcium, however, increased the size of the nodules significantly. The lessened number of nodules per plant, when calcium was added to the inoculating soils, suggests possible death to the organisms by this salt treatment, though this is not significant enough to reduce the percentage of plants inoculated.

On fields other than the Marion Silt Loam, the culture used alone was successful without special treatment. However, in many cases the addition of the calcium, especially the hydroxide, which distributed itself more thoroughly on account of its fineness, gave increased nodules per plant, and increased the percentages of infection. On these fields the color differences were less pronounced than on the Marion Silt Loam, but yet significant differences in growth were evident, as is shown in plate 3.

Determinations of the hydrogen-ion concentration revealed a pH of 6.0 on the Marion Silt Loam where the calcium was beneficial to inoculation, and a much lower figure for the pH where calcium was less effective. Just what relations exist between the hydrogen-ion concentration and the effectiveness of the applied calcium, or between the effectiveness and the electrolysable base or calcium, is still a question. The total electrolysable base and electrolysable calcium content seem to decrease as the calcium additions were less effective.

Part VII

In an attempt to determine the relation of dialyzable base or calcium of the soil to inoculation, the flat phase of the Putnam Silt Loam of the field trials was used in the greenhouse. Seedlings were started for 10 days in calcium-deficient and calcium-laden substrates and then transplanted to this soil given no treatment, given calcium carbonate equivalent to 5 tons per acre, and given calcium chloride at the rate of 1 part per 1500 parts of soil solution, considering the soil at 25 per cent moisture. Thorough inoculation was applied at planting and the plants were grown for 5 weeks, when they were examined for their nodulation. Analyses were made of the seedlings for their calcium content, and of the soil for the total electrolyzable base and calcium. The hydrogen-ion concentration was also determined. The complete data are given in table 8.

Though no statistical manipulation was undertaken to express the reliability of the data in the usual way, it is interesting to note that even though this soil gave no great improvements in its inoculation through calcium applied in the field trials, a significant increase occurred in the nodule numbers when the seedlings carried a liberal calcium supply. This difference was obliterated when the soil was given calcium, either as carbonate or as chloride. No correlation seemed to exist between the electrolysable calcium and the nodule numbers per plant. However, it is interesting to note that the insoluble

calcium carbonate was less effective in increasing nodule numbers per plant, than was the soluble calcium chloride or calcium within the seedlings, for the short time of this trial.

Measurements of the electro-dialyzable calcium within the soils in this study were not numerous enough to establish whether or not this quantity of the element might serve as a possible indication of the soil's deficiency in calcium with reference to inoculation. Further data of this kind will be necessary to decide the question fully. However, the data thus far suggest that electro-dialysis is scarcely a criterion as to whether or not the soil will yield sufficient calcium to guarantee thorough inoculation or whether added calcium might

TABLE 8
Nodule production by soybeans on acid Putnam Silt Loom

| SOIL AND TREATMENT | UNTREATED* | | ONE PART CaCO_3 PER 200 SOIL | | ONE PART CaCl_2 PER 1,500 SOIL SOLUTION† | |
|--|------------|---------|--|---------|--|---------|
| | None | Calcium | None | Calcium | None | Calcium |
| Seedling treatment..... | 73 | 61 | 62 | 79 | 70 | 72 |
| Number of plants..... | | | | | | |
| Average number of nodules per plant..... | 7.9 | 12.8 | 8.0 | 7.3 | 11.9 | 11.0 |
| Range in number of nodules per plant..... | 0-23 | 1-26 | 0-19 | 0-20 | 0-34 | 1-41 |
| Number of plants not inoculated.... | 1 | 0 | 2 | 2 | 1 | 0 |
| Calcium content of plants at replant- ing: mgm. Ca per plant..... | 17.07 | 30.14 | 17.07 | 30.14 | 17.07 | 30.14 |
| Total electro-dialyzable base in soil at end of plant growth: titrable milli- equivalents of base..... | 0.78582 | | 2.15754 | | 0.83412 | |
| Total electro-dialyzable calcium in soil at end of plant growth: mgm. Ca per 10 gm. H_2O -free soil..... | 12.9 | | 41.6 | | 13.4 | |
| pH of soil at end of plant growth..... | 5.82 | | 7.40 | | 5.25 | |

* Original pH of soil—5.14.

† Porosity of treated soil—48.7 per cent. Calculations of soil solution were made for 25 per cent moisture.

improve the establishment of the relation between nodule bacteria and their host plant. Under certain conditions, certainly, very small amounts of calcium are beneficial in establishing thorough inoculation and consequently the legume crop itself. This possibility might be inferred from the work of McCool (11) and by the report of Jaeger (8). How small this amount may be in any case is still a question. Attention may need to be given to ionizable calcium or some other forms before soil analysis can contribute a simple chemical answer to this complex question of biological behavior.

SUMMARY AND CONCLUSIONS

1. The study reported herewith suggests that the beneficial effects of liming for establishing thorough inoculation of legumes on acid soils may be due in part to the element calcium as well as to a change in the degree of acidity.

2. The use of calcium carbonate on an acid soil already well inoculated with *B. radicicola* of soybeans, gave decided improvement in the inoculation of this crop.

3. The addition of calcium chloride to an acid soil, sterile to the soybean organism, favored its longevity from the time of introduction, and improved inoculation on the later planting.

4. A part of the root system of soybean growing in calcium-bearing soil had better inoculation than the part of the same root system growing in calcium-deficient soil. This effect was, then, not readily transmitted to roots in environment deficient in calcium but supplied with the necessary organisms.

5. A liberal supply of calcium within 10-day-old soybean seedlings improved their inoculation when they were transplanted into acid soils.

6. The soybean organisms in colloidal clay suspensions were carried down when flocculated with calcium chloride but not significantly when flocculated with potassium chloride.

7. Field trials found very small quantities of calcium, applied as different salts, a very effective help in increasing inoculation on certain soils and scarcely significant on others.

8. The effect of calcium in stimulating inoculation failed to show a significant correlation to the hydrogen-ion concentration, or the electro-dialyzable calcium in the soil in the few cases studied.

9. Though thorough inoculation may be stimulated in some cases by the addition of calcium, many factors, as fertility of soil and cultural practices are also of significance.

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PLATE 1

SOYBEAN NODULES BY INOCULATION WITH BACTERIA IN COLLOIDAL CLAY

- FIG. 1. Distilled water suspension.
- FIG. 2. Calcium chloride supernatant.
- FIG. 3. Calcium chloride flocculant.
- FIG. 4. Colloidal clay suspension.
- FIG. 5. Potassium chloride supernatant.
- FIG. 6. Potassium chloride flocculant.



1



2



3



4



5



6

PLATE 2

DIFFERENCE IN COLORATION AND NODULATION OF SOYBEANS DUE TO CALCIUM TREATMENT

FIG. 1. Differences in color in consequence of calcium treatment (Marion Silt Loam). Left, cultures and calcium chloride. Right, cultures only.

FIG. 2. Nodulation differences in consequence of calcium treatment. (Marion Silt Loam.) Above, cultures and calcium. Below, cultures only.



FIG. 1

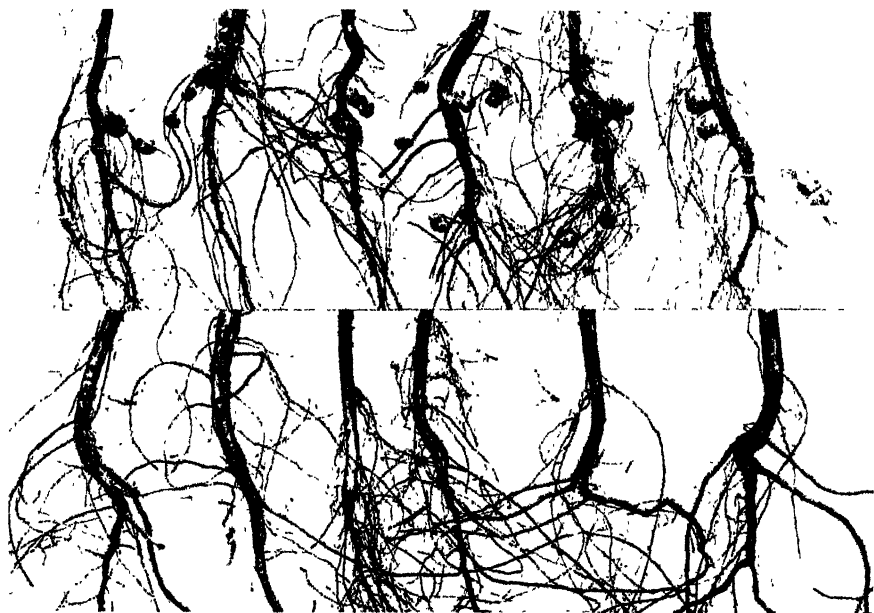


FIG. 2

PLATE 3

SOYBEAN GROWTH ON PUTNAM SILT LOAM AS INFLUENCED BY CALCIUM TREATMENTS

From left, culture, no treatment, culture and acid phosphate, culture and 3 tons limestone



DECOMPOSITION OF CITRIC ACID BY SOIL

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It is well recognized that most soils "fix" the phosphate ions added in the form of water-soluble phosphates, and that the soil solution is poor in phosphorus. On the other hand, many soils have the property of yielding water extracts with about the same content of phosphates to many successive extractions. Some simple laboratory method of measuring quickly the amount of phosphates which the soil will readily supply would be of great utility. For many years the method proposed by Dyer (1) has been widely used for the determination of "available" phosphates in soils. This method consists in extracting the soil with a 1 per cent solution of citric acid under conditions allowing a thorough interaction of solvent and soil with subsequent analysis of the extract. Dyer's original idea was that the acidity of this solvent closely resembled that of the cell sap of roots. His method has persisted, albeit the acidity of the cell contents of roots now appears irrelevant.

In the earlier procedure 200 gm. of air-dry soil is placed in a bottle with 2 liters of water containing 20 gm. of citric acid, and the mixture allowed to remain in contact for a week with many daily shakings, so that some 400 shakings in all are given. The solution is subsequently filtered off and analyzed for various ions, notable potassium and phosphate. We have employed the modified method in which 150 gm. of soil and 1500 cc. of 1 per cent citric acid solution are placed in 2-liter bottle, the stopper secured, and the bottle tumbled for 6 hours in a mechanical shaking device.

It has been somewhat generally accepted that any soil yielding less than 0.01 per cent of P_2O_5 by this citric acid method is in need of phosphatic fertilizers (3). The results of the analyses of 272 Hawaiian soils used for pineapple growing are shown in the graph. It is quite evident that soils analyzing as high as 100 p.p.m., (0.01 per cent) are distinctly uncommon. The mean is 29.4 p.p.m., less than one-third of Dyer's minimum. Our soils, however, give no such general indications of phosphate deficiency as this comparison would indicate.

In the course of a study of the behavior of phosphatic fertilizers in the soil of several representative pineapple fields we have found reasons seriously to question the applicability of the citric acid method.

The question being studied involved the difference in available phosphate which would be found in a given soil following the addition of equivalent amounts of phosphate in the form of finely ground raw rock on the one hand

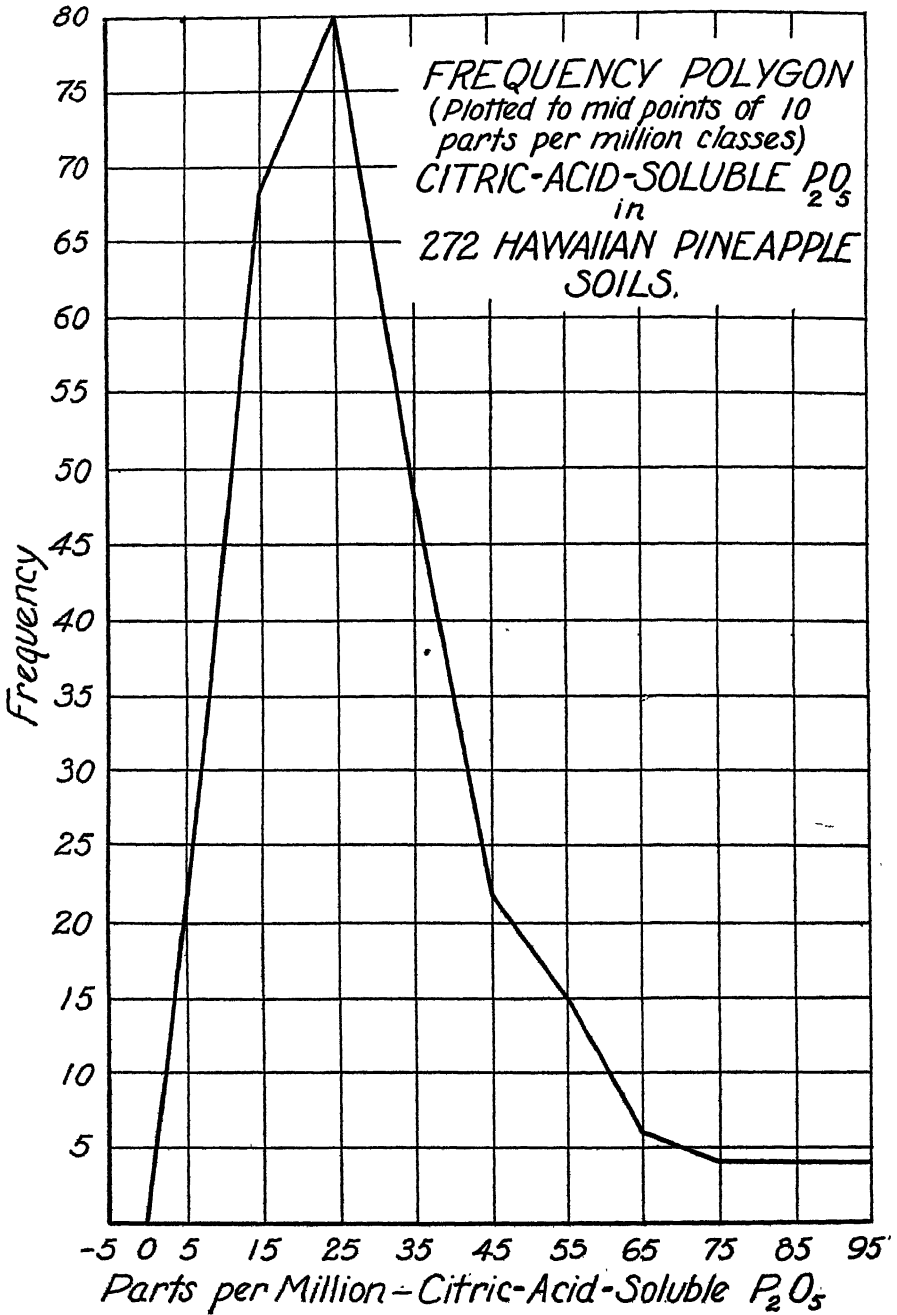


FIG. 1

and of superphosphate on the other. Thirty pounds of soil from pineapple fields in three representative districts on the island of Oahu were carefully mixed with the calculated amounts of the phosphates and placed in tubs. The amounts of the fertilizers corresponded to 1821 pounds of raw rock and 2810 pounds of superphosphate per acre, equivalent to 200 p.p.m. of water-soluble P_2O_5 from superphosphate and the same amount of total P_2O_5 from raw rock, calculated on a basis of 3,000,000 pounds of soil per acre. The moisture content of the soil was raised to about 30 per cent (the moisture equivalent of these soils is around 35 per cent) and the phosphates and soils were allowed to react.

| SOIL | PHOSPHATE ADDED | CITRIC-SOLUBLE P_2O_5 | |
|---------------|-----------------|-------------------------|---------------|
| | | After 7 days | After 14 days |
| | | <i>p.p.m.</i> | <i>p.p.m.</i> |
| H. P. 48 | None | 80 | 68 |
| | Raw rock | 149 | 138 |
| | Superphosphate | 143 | 143 |
| C. P. C. 123 | None | 55 | 57 |
| | Raw rock | 124 | 108 |
| | Superphosphate | 128 | 103 |
| A. H. P. C. 1 | None | 21 | 20 |
| | Raw rock | 23 | 26 |
| | Superphosphate | 33 | 36 |

The results obtained indicate a sharp difference in behavior between two of the soils and the third one. Whereas the addition of phosphates caused a marked increase in the citric-soluble phosphate of the first two, the last was but slightly affected. This last soil is one of the high manganese soils characteristic of certain areas devoted to pineapple growing. A test was made of two other soils containing manganese equivalent to more than 3 per cent Mn_2O_4 . One of these showed a rise of 75 p.p.m. of citric-acid-soluble P_2O_5 following the addition of 200 p.p.m. in the form of superphosphate, the other showed a rise of 22. This indicated no consistent behavior on the part of the high manganese soils.

However little virtue one can see in the use of 1 per cent citric acid because it approximates the acidity of the sap of roots, it does have certain features to recommend it. Citric acid is a slightly dissociated acid and as a consequence a substantial portion of the acid used in the extraction could be neutralized, and the pH of the resulting solution not materially affected. It would, therefore, be rational to assume that by using 1 per cent citric acid one was employing an extracting medium calculated to maintain a mild acidity within narrow limits. Teakle (4) has pointed out the marked differences in solubility of the relatively insoluble soil phosphates due to variations in the pH of the solvent water. To get comparable results with various soils, it is,

therefore, highly desirable that they be extracted with a solvent which will maintain a narrow range of pH in contact with all soils, unless, indeed, one wishes to allow for the effect of the natural pH of the soil and therefore uses distilled water. Our confidence in the uniformity of the pH of citric acid extractions was considerably shaken when determinations were run on two samples and they were found to be far from the 2.2 value of a 1 per cent citric acid solution.

As a result samples from representative pineapple fields on the island of Oahu and one from Molokai were treated with 1 per cent citric acid in accordance with the regular procedure and at the end of the period of shaking the pH of each was determined. The results follow:

| <i>Field</i> | | <i>pH of citrated acid extract</i> |
|----------------------------|----------------|------------------------------------|
| H. P. Co. | 3 C..... | 2.9 |
| | 48..... | 3.7 |
| | 49..... | 4.6 |
| | 24 A..... | 2.3 |
| | 75..... | 6.0 |
| | 76..... | 4.1 |
| | 59 A..... | 3.6 |
| C. P. C. | 139..... | 3.9 |
| | 123..... | 4.0 |
| Libby | 40 C..... | 2.9 |
| | 107..... | 3.0 |
| | Molokai 1..... | 3.1 |
| A. H. P. C. Station 1..... | | 6.0 |

These results show an extraordinary variation. Instead of extracting all soils at uniform pH we have been doing nothing of the sort. The results of 1 per cent citric acid analyses of different soils are not comparable.

We undertook to find out how strong citric acid solution would be required to get a low pH with the Station Field 1 soil, and how much difference the increased acid would make in the dissolved phosphate.

| <i>Solvent</i> | <i>pH</i> | <i>P₂O₅ extracted per cent</i> |
|-----------------------------|-----------|--|
| 1 per cent citric acid..... | 6.0 | 0.0012 |
| 2 per cent citric acid..... | 5.5 | 0.0037 |
| 4 per cent citric acid..... | 3.8 | 0.0169 |
| 8 per cent citric acid..... | 2.9 | 0.0339 |

It was noted that a very high gas pressure develops in the bottles in which some soils are shaken with citric acid. This was especially noticeable in the series just reported—in fact a mud shower bath attended the opening of one of them. A test of the gas showed it to be carbon dioxide. That this was not due to the decomposition of carbonates was shown by the fact that when this same soil was shaken with 1 per cent hydrochloric acid no gas was formed and the pH of the acid remained unchanged. The apparent conclusion is that citric acid is decomposed by the soil with the formation of carbon dioxide as one of the products.

The Hawaiian soils, aside from some few which are of coral origin, have developed through the degradation of basaltic lavas and ashes high in iron. The so-called lateritic soils of tropical regions are reported to be high in ferric oxides. The color of many of the Hawaiian soils is decidedly red. The paper of Fry and Gerwe (2) dealing with the decomposition of citric acid in the presence of light and iron salts was suggestive. They found that in the presence of ferric salts and light, citric acid decomposes to form acetone and carbon dioxide.

To avoid the possibility of the evolution of CO_2 due to biological activities in the soil, two samples of soil of 40 gm. each contained in Erlenmeyer flasks were sterilized in the autoclave and mixed with 400 cc. of water containing in one flask 4 gm. of citric acid and in the other an equivalent amount of hydrochloric acid. The flasks were provided with two-hole stoppers and a gentle stream of air which had passed through KOH was aspirated through the contents of the flasks and then, after being dried, through a CO_2 absorbing bulb. The weight of CO_2 evolved with the citric acid in four hours was .4767 gm., with hydrochloric acid it was .0016 gm.

The citric acid solution which had been shaken with soil from Field 1 of the Station in the regular analytical procedure was distilled and the distillate tested for acetone by Gunning's test. A yellow precipitate and the characteristic odor of iodoform were readily obtained. A similar distillate was prepared and tested for acetone by the preparation of dibenzylacetone (melting point, 110° – 112°C .). The product obtained melted at 109.5° , and a similar product obtained from pure acetone melted at 108° . The mixture of the two melted at 108.5° . When the experiment was repeated using for the shaking a bottle painted black, gas was evolved and a sample of dibenzylacetone melting at 108.5° obtained from the distillate.

From the results just detailed we are convinced that some of our Hawaiian soils have the property of decomposing citric acid solutions to such a degree as seriously to affect the value of this solvent. Two of the products of decomposition are carbon dioxide and acetone, which, in the light of the work of Fry and Gerwe, are presumptive evidence of the activity of the iron compounds of the soil.

As a partial check on the phosphate-supplying power of the three soils reported on in the preceding, five tubs were filled with each soil, phosphates well mixed in according to the schedule, and cowpeas planted. Fifteen plants were allowed to grow in each tub for 90 days. The appearance of the plants at the end of the growing period is shown in plate 1, and the analytical results are listed in the table on following page.

There is no consistent relation between the amount of phosphorus the cowpeas could remove and that extracted by 1 per cent citric acid. On the other hand, the soil yielding the lowest citric acid extract furnished the most phosphate to the plants, and at the same time had such a destructive action on citric acid that the pH of its 1 per cent solution was raised to 6.0 during the analytical extraction. The low citric-soluble phosphate of our group of Hawaiian pineapple soils is very possibly related to the loss of acidity of the citric acid during

the procedure. However valuable the Dyer method may be as a measure of "available" phosphate in soils in other countries, we are convinced that it is wholly unreliable under our conditions.

| FIELD | TOTAL P_2O_5 IN ORIGINAL SOIL | FERTILIZER | | CITRIC- SOLUBLE P_2O_5 | DRY WEIGHT COWPEAS PER TUB | P_2O_5 PER TUB |
|--------------|---------------------------------------|------------|---------------|--------------------------------|----------------------------------|------------------|
| | | Kind | P_2O_5 | | | |
| | <i>per cent</i> | | <i>p.p.m.</i> | <i>p.p.m.</i> | <i>gm.</i> | <i>gm.</i> |
| H. P. Co. 48 | 0.284 | None | None | 70 | 41 | 0.111 |
| | | Raw rock | 200 | 140 | 43 | 0.109 |
| | | Raw rock | 400 | —* | 47 | 0.125 |
| | | Super | 200 | 143 | 62 | 0.197 |
| | | Super | 400 | —* | 72 | 0.326 |
| C. P. C. 123 | 0.265 | None | None | 56 | 17 | 0.057 |
| | | Raw rock | 200 | 116 | 48 | 0.151 |
| | | Raw rock | 400 | —* | 52 | 0.179 |
| | | Super | 200 | 115 | 53 | 0.213 |
| | | Super | 400 | —* | 55 | 0.250 |
| Station 1 | 0.362 | None | None | 20 | 50 | 0.211 |
| | | Raw rock | 200 | 24 | 53 | 0.228 |
| | | Raw rock | 400 | —* | 41† | 0.244 |
| | | Super | 200 | 34 | 55 | 0.326 |
| | | Super | 400 | —* | 86 | 0.386 |

* Not determined.

† 14 plants only.

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- (3) RUSSELL, E. J. Soil Conditions and Plant Growth, ed. 5, p. 371.
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PLATE 1

COWPEAS

From top to bottom: H. P. Co. Field No. 48

C. P. C. Field No. 123

A. H. P. C. Field No. 1

Right to left: 1. No treatment

2. Raw rock 200 p.p.m. P_2O_5

3. Raw rock 400 p.p.m. P_2O_5

4. Superphosphate 200 p.p.m. P_2O_5

5. Superphosphate 400 p.p.m. P_2O_5



THE RELATION BETWEEN THE ABSORBED AND THE EXCHANGEABLE CALCIUM AND MAGNESIUM CONTENT OF A SOIL FOUR YEARS AFTER ADDITIONS

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The subject of exchangeable bases in the soil has been dealt with in numerous articles during recent years. Special stress has been placed upon the replaceability of calcium. A number of methods and modifications have been advanced to differentiate between those bases and alkaline-earths that have passed into combination with the clay-humus complexes and those that are still present in mineral, or non-exchangeable, form.

The contributions hitherto made have been mainly studies upon the bases that were native to the soil. It is of interest and value to study the availability of the calcium that has withstood the agencies of weathering; but, if liming is necessary, the fate of added liming materials is even more important, since the availability, or replaceability, of the absorbed calcium and magnesium that is derived from economic additions is probably a primary factor in any sustained benefit from liming.

EXPERIMENTAL

The plan of the present study was to determine the replaceable, or exchangeable, calcium and magnesium content of a soil that had absorbed or "fixed" known quantities (12) from certain additions that had been made four years previously in outdoor lysimeter studies. The method of Hissink (5) was decided upon as being a recognized procedure for the determination of exchangeable bases.

The soil used was a brown loam that had been under control and subject to outdoor conditions in $\frac{1}{20,000}$ -acre lysimeters from 1921 to 1925. Thirteen different calcic and magnesian treatments of constant CaO equivalence had been made in the upper and also in the lower zones, or halves. The two zones were demarcated by a wire-cloth disc. In the series where the treatments had been incorporated only with the upper half of the 8-inch depth of soil, the percolates had passed through an underlying untreated layer. In a parallel series the upper zone had received no additions and the percolates from the lower treated zone had passed directly into the percolate receptacles. The fraction of each addition that was still present as a non-hydroxide and non-carbonate compound was designated as that which had been absorbed

or "fixed" by the soil. This fraction was determined by subtracting from the CaCO_3 -equivalence of each treatment the sum of its carbonate residue and calcium + magnesium outgo.

At the end of the four years of exposure each zone of each tank was removed, thoroughly mixed, and air-dried, and a 2-quart sample preserved. Charges of 25 gm. of a 2-mm. sieving were used to determine the calcium and magnesium extracted by the leachates of normal sodium chloride. Parallel preliminary extractions were also made with solutions of normal ammonium chloride. The ammonium chloride solution exerted greater solvent action upon those particles of high-calcic and dolomitic limestone that still remained where the coarser separates had been added. This is in harmony with Hissink's observations (5) relative to the solvent action exerted by the two chlorides upon calcium carbonate. The different caustic, limestone, and dolomite treatments are given in each table, along with the calcium-magnesium data that are reported on a basis of CaCO_3 equivalence.

THE RECOVERIES OBTAINED BY THE HISSINK PROCEDURE

Unlimed controls

Comparisons between the Hissink-method data are made against the average of the controls that had been exposed for four years and also against the original soil that had been preserved, under seal, in the laboratory. The data of table 1 show a decrease of 939 pounds of exchangeable calcium + magnesium for the full depth of unsupplemented soil for the 4-year period. This decrease was accounted for by a loss of 353 pounds from the upper half, and 586 pounds from the lower half. The total calcium-magnesium that had been found in the percolates from the control tanks during this period was 1234 pounds—898 pounds attributable to calcium and 336 to magnesium (7). This represents a ratio of 2.67:1 in the outgo, whereas extractions from the reserve sample of the original soil gave, by the Hissink method, an exchangeable calcium-magnesium ratio of 5.68 to 1. The corresponding ratios for the upper and lower zones of the exposed soil after four years, without additive treatment, were 5.32:1 and 7.75:1, respectively. Hence, the exchangeable calcium-magnesium was apparently 395 pounds less than the actual outgo of the two alkaline-earth bases; but the analysis of rain-gauge waters showed that the rainfall had supplied 296 pounds of soluble calcium-magnesium salts. It is therefore evident that there is a marked difference between the ratio of calcium to magnesium in the natural percolates, and that found in the sodium chloride leachates from the unlimed soil. The proportions of magnesia in the natural leachings are much greater than those found in the sodium chloride extractions.

The decrease in the exchangeable calcium-magnesium content of the lower zone of the exposed controls was considerably greater than that shown by the upper zone, in a comparison with the original soil. This evidence of

The exchangeable calcium + magnesium present in a naturally leached brown loam four years after additions of various liming materials to the surface zone, as determined by the Hissink Method*—terms of CaCO_3 equivalence

| TANK NUMBER | TREATMENTS EQUIVALENT TO 3570 POUNDS CaCO_3 PER ACRE | SOIL THAT RECEIVED THE ADDITIONS | | | | | | SOIL THAT UNDERLAY THE TREATED SOIL | | | | | | FULL DEPTH BOTH ZONES | | |
|----------------|--|----------------------------------|-------|------------|--|------------------|---------------------|-------------------------------------|-------|------------|---|------------------|---------------------|--|------------------|---------------------|
| | | Analysis air-dry soil | | | Pounds Ca + Mg upper zone or half, 1,000,000 pounds moisture-free soil | | | Analysis air-dry soil | | | Pounds Ca + Mg upper zone or half, 1,000,000 pounds moisture-free soil | | | Pounds Ca + Mg per 2,000,000 pounds moisture-free soil | | |
| | | Ca | Mg | Ca + Mg | Found | Original soil | Exposed controls | Ca | Mg | Ca + Mg | Found | Original soil | Exposed controls | Found | Original soil | Exposed controls |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| per cent | per cent | per cent | 2,617 | —353 | | per cent | per cent | per cent | 0.236 | —586 | ... | 5,001 | —939 | | | |
| 80 A + B | Controls | 0.394 | 0.046 | 0.440 | 4,445 | 1,475 | 1,828 | 0.291 | 0.023 | 0.314 | 3,172 | 202 | 788 | 7,617 | 1,677 | 2,616 |
| 84 | Hydrated Lime | 0.295 | 0.127 | 0.422 | 4,263 | 1,293 | 1,646 | 0.240 | 0.077 | 0.317 | 3,203 | 233 | 819 | 7,466 | 1,526 | 2,465 |
| 85 | Burnt Dolomite | 0.303 | 0.125 | 0.428 | 4,324 | 1,354 | 1,707 | 0.237 | 0.081 | 0.318 | 3,213 | 243 | 829 | 7,537 | 1,597 | 2,536 |
| 86 | Dolomite-oxide mixture | 0.367 | 0.045 | 0.412 | 4,162 | 1,192 | 1,545 | 0.228 | 0.040 | 0.268 | 2,708 | 262 | 324 | 6,870 | 930 | 1,869 |
| 88 | Limestone, 10-20 mesh | 0.393 | 0.044 | 0.437 | 4,415 | 1,445 | 1,798 | 0.273 | 0.029 | 0.302 | 3,051 | 81 | 667 | 7,466 | 1,525 | 2,465 |
| 89 | Limestone, 20-40 mesh | 0.403 | 0.035 | 0.438 | 4,425 | 1,455 | 1,808 | 0.286 | 0.025 | 0.311 | 3,142 | 172 | 758 | 7,567 | 1,627 | 2,566 |
| 90 | Limestone, 40-80 mesh | 0.385 | 0.040 | 0.425 | 4,294 | 1,324 | 1,677 | 0.307 | 0.018 | 0.325 | 3,283 | 313 | 899 | 7,577 | 1,637 | 2,576 |
| 91 | Limestone, 80-200 mesh | 0.367 | 0.050 | 0.417 | 4,213 | 1,243 | 1,596 | 0.264 | 0.024 | 0.288 | 2,910 | —60 | 526 | 7,123 | 1,183 | 2,122 |
| 87 | Limestone, Composite† | 0.273 | 0.100 | 0.373 | 3,768 | 798 | 1,151 | 0.215 | 0.036 | 0.251 | 2,536 | —434 | 152 | 6,304 | 364 | 1,303 |
| 93 | Dolomite, 10-20 mesh | 0.300 | 0.106 | 0.406 | 4,102 | 1,132 | 1,485 | 0.232 | 0.051 | 0.283 | 2,859 | —111 | 475 | 6,961 | 1,021 | 1,960 |
| 94 | Dolomite, 20-40 mesh | 0.319 | 0.114 | 0.433 | 4,375 | 1,405 | 1,758 | 0.242 | 0.061 | 0.303 | 3,061 | 91 | 677 | 7,436 | 1,496 | 2,435 |
| 95 | Dolomite, 40-80 mesh | 0.322 | 0.111 | 0.433 | 4,375 | 1,405 | 1,758 | 0.250 | 0.061 | 0.311 | 3,142 | 172 | 758 | 7,517 | 1,577 | 2,516 |
| 96 | Dolomite, 80-200 mesh | 0.292 | 0.109 | 0.401 | 4,051 | 1,081 | 1,434 | 0.230 | 0.052 | 0.282 | 2,849 | —121 | 465 | 6,900 | 960 | 1,899 |
| 92 | Dolomite, Composite† | 0.250 | 0.044 | 0.294 | 2,970 | | | | | | | | | 5,940 | | |
| | Unexposed soil, preserved air-dry | | | | | | | | | | | | | | | |

* Using Normal NaCl.

† Composed of equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

greater loss from the lower zone is in harmony with findings relative to a more intensive evolution of CO_2 in the lower zone (15), which is usually more moist.

The replacement of magnesium by sodium was practically constant for these upper-zone units to which no magnesium was added. The replacements from the lower-zone units, however, were less than those from the controls, save for the coarsest limestone separate of 10-20-mesh. This may be due to the previous replacement and leaching of magnesium by the neutral calcium salts that had been engendered in the upper zone of treatment during the four years of exposure (7, 8).

Surface-zone additions

The data of table 1 show the enhancements that were registered by the Hissink method for the limed upper zone and also those found in the underlying one. Every surface-zone addition caused an increased supply of exchangeable calcium-magnesium, on the basis of full depth of soil, in comparison with both the original soil and the exposed controls. This was not true, however, in a comparison between the underlying untreated zone and the original soil. Each underlying layer showed an increased calcium-magnesium content over that of the lower zone of the exposed controls, but the increments brought by percolates from the less rapidly disintegrated, coarsest limestone separate and composite were insufficient to offset the difference between the sum of the exchangeable calcium and magnesium originally present and that found in the underlying untreated zone after four years. This held also for the 10-20-mesh, 20-40-mesh, and composite dolomite additions. The average ratio between the enhanced amounts of calcium-magnesium for the upper zone that was in direct contact with the surface-zone additions and those enhancements found for the lower zone was 2.6 to 1. In the eight instances where increments to the underlying zone were larger than the respective amounts lost from the untreated soil during the 4-year period there was an average increase of 188 pounds, whereas the corresponding enhancement average for the upper zone was 1395 pounds. This gives an upper-zone to lower zone ratio of 7.4 to 1. For the full depth of soil the 13 additions showed an average gain of 1317 pounds in exchangeable alkaline-earth bases, in comparison with the original soil, and 2256 pounds against the exposed soil. The enhancements derived from the equivalent additions of high-calcic and dolomitic limes, 20-40-, 40-80-, and 80-200-mesh limestones, and the 40-80-mesh dolomite separates are in close agreement. The tendency of the soil to absorb somewhat more limestone than dolomite, or rather to absorb the former more rapidly, is shown by comparisons between the two limestone groups in the last column. This differential is at a minimum, however, in the case of the 80-mesh products.

Subsurface-zone additions

In table 2 are given the recoveries of exchangeable calcium and magnesium from the additions to the lower zone. Previous studies and observations with these tanks warrant the assumption that the several units of the upper untreated zone may be considered as a constant. In this series there was no catch zone and the calcium-magnesium losses through leaching had been consistently greater (7, 8) than those from the series where additions were made

TABLE 2

The exchangeable calcium + magnesium present in a naturally leached brown loam four years after additions of various liming materials to the subsurface zone, as determined by the Hissink Method—terms of CaCO₃ equivalence

| TANK NUMBER | TREATMENTS EQUIVALENT TO 3570 POUNDS CaCO ₃ PER ACRE | ANALYSIS AIR-DRY SOIL | | | Ca + Mg IN ZONE, MOISTURE-FREE BASIS | | |
|-------------|---|-----------------------|-----------------|-----------------|--------------------------------------|---------------|------------------|
| | | Ca | Mg | Ca+Mg | Found | Increase over | |
| | | | | | | Original soil | Leached controls |
| | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>pounds</i> | <i>pounds</i> | <i>pounds</i> |
| 80 A + B | Controls | 0.209 | 0.027 | 0.236 | 2,384 | -586 | |
| 84 | Hydrated Lime | 0.416 | 0.023 | 0.439 | 4,435 | 1,465 | 2,051 |
| 85 | Burnt Dolomite | 0.341 | 0.092 | 0.433 | 4,375 | 1,405 | 1,991 |
| 86 | Dolomite-oxide mixture | 0.352 | 0.091 | 0.443 | 4,476 | 1,506 | 2,092 |
| 88 | Limestone, 10-20 mesh | 0.406 | 0.028 | 0.434 | 4,385 | 1,415 | 2,001 |
| 89 | Limestone, 20-40 mesh | 0.425 | 0.025 | 0.450 | 4,546 | 1,576 | 2,162 |
| 90 | Limestone, 40-80 mesh | 0.419 | 0.029 | 0.448 | 4,526 | 1,556 | 2,142 |
| 91 | Limestone, 80-200 mesh | 0.409 | 0.028 | 0.437 | 4,415 | 1,445 | 2,031 |
| 87 | Limestone, Composite* | 0.408 | 0.030 | 0.438 | 4,425 | 1,455 | 2,041 |
| 93 | Dolomite, 10-20 mesh | 0.304 | 0.081 | 0.385 | 3,890 | 920 | 1,506 |
| 94 | Dolomite, 20-40 mesh | 0.335 | 0.083 | 0.418 | 4,223 | 1,253 | 1,839 |
| 95 | Dolomite, 40-80 mesh | 0.349 | 0.090 | 0.439 | 4,435 | 1,465 | 2,051 |
| 96 | Dolomite, 80-200 mesh | 0.345 | 0.095 | 0.440 | 4,445 | 1,475 | 2,061 |
| 92 | Dolomite, Composite* | 0.339 | 0.091 | 0.430 | 4,344 | 1,374 | 1,960 |
| | Unexposed Soil | 0.250 | 0.044 | 0.294 | 2,970 | | |

* Composed of equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

to the upper zone. It follows that there was a smaller quantity of residual exchangeable bases to be extracted by the sodium chloride treatment. On the other hand, the disintegration of the limestone and dolomite separates was much more extensive in the lower zone (11); hence, rather uniform values obtained for 12 of the 13 caustic and limestone and dolomite additions, the one exception being the coarsest, or 10-20-mesh, dolomite separate.

The enhancements found by the Hissink method show, for the 13 lower-zone additions, an average of 1385 pounds over the original soil and a corre-

sponding average of 1994 pounds in excess of the final exchangeable calcium-magnesium content of the exposed soil. This average of 1994 pounds was from a series that gave, through natural leaching, a loss of 850 pounds in excess of that from the controls, whereas, in the case of the 13 surface-zone additions the average enhancement of 2266 pounds represented the full-depth effect where the calcium-magnesium loss had been only 155 pounds in excess of that from the controls.

TABLE 3

The exchangeable Ca + Mg, determined by the Hissink Method four years after surface-zone additions of various liming materials, as compared with the "fixed" Ca + Mg determined in lysimeter studies by a balance between additions, outgo, and carbonate residues—terms of CaCO₃ equivalence

| TANK NUMBER | TREATMENT* | EFFECT EXERTED BY BOTH UPPER AND LOWER ZONES | | | | | | |
|-------------|------------------------|--|---|---|--------|--|----------------------|--|
| | | Carbonate residues from additions in soil analyzed | Four-year outgo Ca + Mg over that of controls | Increase in non-carbonate Ca + Mg | | | | Ratio between determined occurrences of the "fixed" and the exchangeable Ca + Mg |
| | | | | Determined as "fixed"† in lysimeter studies | | Determined as exchangeable by the Hissink Method | | |
| | | | | pounds | pounds | pounds | per cent of addition | |
| 84 | Hydrated Lime | 160 | 271 | 3,139 | 87.9 | 2,616 | 73.3 | 1.20 to 1 |
| 85 | Burnt Dolomite | 140 | 231 | 3,199 | 89.6 | 2,465 | 69.0 | 1.30 to 1 |
| 86 | Dolomite-oxide mixture | 140 | 156 | 3,274 | 91.7 | 2,536 | 71.0 | 1.29 to 1 |
| 88 | Limestone, 10-20 mesh | 1,670 | 78 | 1,822 | 51.0 | 1,869 | 52.4 | 0.97 to 1 |
| 89 | Limestone, 20-40 mesh | 270 | 170 | 3,130 | 87.7 | 2,465 | 69.0 | 1.27 to 1 |
| 90 | Limestone, 40-80 mesh | 40 | 211 | 3,319 | 93.0 | 2,566 | 71.9 | 1.29 to 1 |
| 91 | Limestone, 80-200 mesh | 100 | 226 | 3,244 | 90.9 | 2,576 | 72.2 | 1.26 to 1 |
| 87 | Limestone, Composite‡ | 650 | 51 | 2,869 | 80.4 | 2,122 | 59.4 | 1.35 to 1 |
| 93 | Dolomite, 10-20 mesh | 1,970 | 38 | 1,562 | 43.8 | 1,303 | 36.5 | 1.20 to 1 |
| 94 | Dolomite, 20-40 mesh | 870 | 105 | 2,595 | 72.7 | 1,960 | 54.9 | 1.32 to 1 |
| 95 | Dolomite, 40-80 mesh | 510 | 152 | 2,908 | 81.5 | 2,435 | 68.2 | 1.19 to 1 |
| 96 | Dolomite, 80-200 mesh | 150 | 207 | 3,213 | 90.0 | 2,516 | 70.5 | 1.28 to 1 |
| 92 | Dolomite, Composite‡ | 1,040 | 115 | 2,415 | 67.6 | 1,899 | 53.2 | 1.27 to 1 |

* Each treatment = 3570 pounds of CaCO₃.

† The CaCO₃-equivalent addition minus the sum of the carbonate increase and enhancement in outgo.

‡ Composed of equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

COMPARISON BETWEEN HISSINK-METHOD RESULTS AND LYSIMETER ABSORPTION DATA

Surface-zone additions

The data of table 3 give comparisons between the amounts of calcium-magnesium that had been absorbed or "fixed" by the two zones of full soil

CALCIUM AND MAGNESIUM CONTENT OF SOIL

depth from the 13 upper-zone additions and the corresponding increases in the exchangeable calcium-magnesium registered by the Hissink procedure. From the constant 3570-pound equivalence of CaCO_3 (2000 pounds CaO) was deducted the respective sums of carbonate increases and enhanced outgo, in order to secure the data of the third column. The "fixed," or absorbed, data are higher than the Hissink-method values, without exception. The

TABLE 4

The exchangeable Ca + Mg, determined by the Hissink Method four years after subsurface additions of various liming materials, as compared with the "fixed" Ca + Mg determined in lysimeter studies by a balance between additions, outgo, and carbonate residues—terms of CaCO_3 equivalence

| TANK NUMBER | TREATMENT* | EFFECT EXERTED BY A CONSTANT UNTREATED SURFACE ZONE AND THE LOWER ZONE OF ADDITIONS | | | | | | | |
|-------------|------------------------|---|---|---|--------|--|----------------------|--|--------|
| | | Carbonate residues from additions in soil analyzed | Four-year outgo Ca + Mg over that of controls | Increase in non-carbonate Ca + Mg | | | | Ratio between determined occurrences of the "fixed" and the exchangeable Ca + Mg | |
| | | | | Determined as "fixed"† in lysimeter studies | | Determined as exchangeable by the Hissink Method | | | |
| | | | | pounds | pounds | pounds | per cent of addition | | pounds |
| 84 | Hydrated Lime | 140 | 1,148 | 2,282 | 63.9 | 2,051 | 57.5 | 1.11 to 1 | |
| 85 | Burnt Dolomite | 200 | 1,215 | 2,155 | 60.4 | 1,991 | 55.8 | 1.08 to 1 | |
| 86 | Dolomite-oxide mixture | 200 | 1,090 | 2,280 | 63.9 | 2,092 | 58.6 | 1.09 to 1 | |
| 88 | Limestone, 10-20 mesh | 860 | 506 | 2,204 | 61.7 | 2,001 | 56.1 | 1.10 to 1 | |
| 89 | Limestone, 20-40 mesh | 170 | 817 | 2,583 | 72.4 | 2,162 | 60.6 | 1.19 to 1 | |
| 90 | Limestone, 40-80 mesh | 000 | 1,098 | 2,472 | 69.2 | 2,142 | 60.0 | 1.15 to 1 | |
| 91 | Limestone, 80-200 mesh | 000 | 1,133 | 2,437 | 68.3 | 2,031 | 56.9 | 1.20 to 1 | |
| 87 | Limestone, Composite‡ | 340 | 900 | 2,330 | 65.3 | 2,041 | 57.2 | 1.14 to 1 | |
| 93 | Dolomite, 10-20 mesh | 1,550 | 153 | 1,867 | 52.3 | 1,506 | 42.2 | 1.24 to 1 | |
| 94 | Dolomite, 20-40 mesh | 620 | 486 | 2,464 | 69.0 | 1,839 | 51.5 | 1.34 to 1 | |
| 95 | Dolomite, 40-80 mesh | 160 | 805 | 2,605 | 73.0 | 2,051 | 57.5 | 1.27 to 1 | |
| 96 | Dolomite, 80-200 mesh | 110 | 1,013 | 2,447 | 68.5 | 2,061 | 57.7 | 1.19 to 1 | |
| 92 | Dolomite, Composite‡ | 700 | 689 | 2,181 | 61.1 | 1,960 | 54.9 | 1.11 to 1 | |

* Each treatment = 3570 pounds CaCO_3 .

† The CaCO_3 -equivalent addition minus the sum of the carbonate increase and enhancement in outgo.

‡ Composed of equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

average ratio between the calcium-magnesium absorption data and the Hissink-method recoveries is 1.24:1, with rather close agreement for 12 of the 13 units. The lysimeter results give an average "fixation," or absorption, of 2822 pounds. This is equivalent to 79.1 per cent of the constant value of the additions, whereas the average enhancement in exchangeable calcium-magnesium by the Hissink procedure was 2178 pounds, or 63.2 per cent.

Subsurface-zone additions

The data of table 4 give corresponding results from the series that received the additions to the lower zone. The lysimeter data show that an average of 2324 pounds, or 65.3 per cent of the 3570-pound CaCO_3 -equivalent additions, was still present in the non-carbonate form, whereas the average of the enhancements registered by the Hissink procedure was 1994 pounds, or 55.9 per cent. The average ratio between the "fixed," or absorbed, bases and the enhancement obtained by the Hissink method is 1.09 to 1. In this zone of greater carbonate decomposition, greater outgo through percolations, and lesser retention, the results by the two methods of attack are more nearly in accord.

GENERAL DISCUSSION

A closer concordance would be expected if both methods truly record the "fixed," or absorbed, fractions of the additions after a period of four years of exposure to weather. It was thought possible that the mechanics of the Hissink procedure might be altered so as to effect a greater recovery and, hence, a closer agreement in the results obtained by the two methods. The extractions were therefore repeated after preliminary dispersion had been effected by means of the electric device proposed by Bouyoucos (3), and modified by Baver (2). Special glass containers with rubber covers and vertical glass baffle rods were used to effect maximum dispersion. The dispersed systems were then filtered as in the regular Hissink procedure. Gravitational filtration was greatly retarded as a result of the preliminary dispersion, but the results for both calcium and magnesium were practically identical with those obtained by the standard procedure. A detailed presentation of these data would represent merely a duplication of those already given. One indication developed from this comparison. Variation in methods of manipulation may appreciably alter the amounts of manganese that will be extracted by the leachates. It is advisable, and may be necessary, to remove this element, along with iron and aluminum, by means of ammonium persulfate, prior to the precipitation of calcium.

It has been assumed that the process used in the Hissink procedure would recover the full amount of the exchangeable bases, calcium more especially. Moreover, the possibility that the NaCl solution might exert an appreciable solvent action upon "acid-soluble" mineral non-complexes has been considered, and by Hissink himself (5, p. 272). He logically contended that any such slight solvent action, and plus error, would not materially influence the value of results obtained by the use of his method. The plus error attributable to the solubility of alkaline-earth carbonates is admitted and provided for (5, p. 271).

The Hissink procedure gives concordant determinations and these evidently register some definite property. This method, and similar procedures, have their place and value, and the principle involved is not being condemned.

The method may truly determine the amount of *native* bases that are components of the exchange complex. It may also give a full recovery of those fractions of the lime additions that enter into true exchangeable or replaceable form. But the Hissink method uniformly failed to extract the full amounts of the non-carbonate residuals that had been derived from a 3570-pound CaCO_3 constant.

On the other hand, the lysimeter values may be considered as being close to the absolute. The neutralizing equivalence of each addition was determined by its titration value, supplemented by CaO and MgO determinations. The calcium-magnesium content of the percolates was determined by precision methods, whereas the carbonate-residue determinations were made by the Tennessee station (9), and official, method (1). This permits the use of 100-gm. charges in an especially adapted shaking device and insures extreme accuracy in the determination of minute amounts of carbonate- CO_2 .

The relationship between the two series of data was somewhat uniform. This may mean one of two things, at least for this particular soil. Either (a) the Hissink method did not extract all of the absorbed or exchangeable calcium and magnesium that had been derived from additions, or (b) a part of the added calcium and magnesium entered into non-exchangeable or exceedingly resistant forms. When considered in the light of related data the second explanation appears more tenable.

Previous studies at the Tennessee station have shown that calcium and magnesium carbonates may react with different non-complex silicic and titanic materials (9, 6). It was especially emphasized that the reaction tendency of silicic acid, even in crystalline form, had received little attention and that it should not be disregarded. It was shown later that the addition of C. P. precipitated silica along with 4-ton additions of MgO resulted in a MgO-SiO_2 reaction and in the eradication of a lethal toxicity (15).

The products that result from reactions between calcium or magnesium and silica may not enter extensively into base-exchange reactions. If the pulverized, simple, natural silicate wollastonite may be used as an index of base-exchange reactions to be expected from combinations that ensue from contact between calcium compounds and colloidal silica, some of the calcium that is fixed by the soil would be expected to resist the solvent action of sodium chloride leachings. This was evidenced when a 5-gm. charge of wollastonite was mixed with 95 gm. of quartz and subjected to the Hissink procedure. In terms of CaCO_3 -equivalence, the calcium content per liter was 22.4 mgm. whereas that of magnesium was 4.1 mgm. Serpentine gave corresponding extractions of 12.4 and 30.9 mgm.

The possibility of such a combination between added calcium compounds and soil components other than the exchange complex has also recently been suggested by Pierre (19) to account for certain observations made by him in a related type of work. In the case of repeated use of soluble potassic manures it has been shown that the added salts retreated into forms that were

not recoverable by base-exchange methods. The work of Frear and Erb at the Pennsylvania station (4), previous Tennessee station results (17), and the studies by Page and Williams (18) indicate very strongly that the continued use of soluble potassic salts results in the accumulation of combinations that are not dissolved out by procedures that are used to determine exchangeable bases.

Furthermore, the process of aging might be expected to decrease the solubility of absorption compounds, even if these were to remain constant in makeup, instead of passing into more complex forms. The factor of progressive decrease in solubility was suggested in previous data from this station (12), and further emphasized through calculations (9, p. 192) that indicated that absorbed calcium-magnesium had become progressively more resistant to percolation by rainwaters.

In the case of the present work, the time factor may be an important one. It seemed possible that the absorbed bases might have been recovered completely by such a procedure as the Hissink method, if the extractions had been made immediately, or shortly, after the absorption had taken place. This point was considered by the following minor experiment. Twenty-five-gram charges of soil were treated with a standard calcium hydroxide solution in an amount equivalent to a rate of 2000 pounds of CaO per 2,000,000 pounds of soil. This addition represented a small fraction of the absorptive capacity of the soil. The suspension was then evaporated to an air-dry condition, in a larger desiccator at room temperature and in a CO₂-free atmosphere. Three days were required for this treatment. The dry samples were then subjected to sodium chloride extractions by the Hissink procedure, in parallel with the untreated soil that had been used in the lysimeter studies. When the calcium that was removed by 2 liters of leachate from the original soil was subtracted from the amount obtained from the lime-treated charges, the recovery was 90 per cent of the addition. The unextracted 10 per cent fraction of the addition, however, amounted to only 0.01 per cent on the basis of soil charge. This tends to confirm the hypothesis that a reaction transpires between added lime and soil components other than the exchange complex.

One other possible factor may be of importance. It has been shown by Steenkamp (20) that the spontaneous dehydration of a soil exerts a marked influence upon its tendency to yield exchangeable bases by extraction methods, and that an increase or decrease is specific for a given soil. The lysimeter soils were spread and dried immediately after their removal from the tanks and kept in the air-dry condition under seal in the laboratory from 1925 to 1928. It is possible that, had the sodium chloride extractions been made upon the freshly taken samples, three years previously, the recoveries of the added calcium and magnesium might have been of different magnitude from those obtained after drying and aging. This possibility is not supported, however, by the results obtained when the Hissink method was used in an attempt to recover the full amount of added Ca(OH)₂ shortly after the addition had been made.

SUMMARY

The Hissink method was used to determine the enhancements in exchangeable calcium-magnesium from surface-zone and subsurface-zone incorporations of high-calcic and high-magnesian limes, limestone, and dolomite separates of a constant 3570-pound CaCO_3 equivalence that had been incorporated four years previously in 28 outdoor lysimeters. These findings were compared with the "fixation," or absorption, results obtained in the lysimeter studies.

The ratio of calcium to magnesium in the sodium chloride leachates from the original soil, and also that from the exposed controls, was decidedly greater than the one found in the rainwater percolates. The decrease in the exchangeable base content of the lower zone of the controls was materially greater than that found for the upper zone.

Each surface-zone addition caused an extraction of exchangeable bases in excess of that obtained from the surface zone of the original soil. This did not hold for the underlying unlimed zone in the case of the less extensively disintegrated limestone and dolomite separates.

The enhancements obtained by the Hissink method were uniform for the additions to the lower zone, where both natural-leaching losses and carbonate disintegration had been the greater.

The enhancements registered by the Hissink method were consistently less than the absorptions. The average "fixation" shown by the lysimeter studies for surface-zone incorporations was 79.1 per cent of the constant addition, whereas the enhancement registered by the Hissink procedure was 63.2 per cent. Corresponding values obtained by the two methods from the subsurface-zone incorporations were 65.3 and 55.9 per cent, respectively.

The average absorption from the 13 surface-zone incorporations was 1.25 times the calcium-magnesium enhancements found by the Hissink method. A corresponding figure of 1.17 was obtained for the subsurface-zone incorporations.

Preliminary dispersion failed to increase the recoveries obtained by the Hissink method.

The uniformly higher values for "fixation," or absorption, in the lysimeter studies indicated that a fraction of the additions had combined with soil components other than the exchange complex. This viewpoint was strengthened by citation of related work, previously reported, and by the incomplete recovery of freshly added Ca(OH)_2 , in controlled atmosphere, by the technic of the Hissink method.

Addenda corrections are given for previous data relative to limestone and dolomite disintegration and "fixation."

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ADDENDA
A CORRECTION IN CALCULATIONS
FOR

"THE DISINTEGRATION OF LIMESTONE AND DOLOMITE SEPARATES, AS
INFLUENCED BY ZONE OF INCORPORATION"¹

AND

"FIXATION OF CALCIUM-MAGNESIUM FROM BURNT LIMES, LIMESTONE
AND DOLOMITE INCORPORATIONS IN TWO SOIL ZONES"²

By W. H. MACINTIRE AND W. M. SHAW

In carrying out the studies reported in the foregoing paper a marked disparity appeared between the results obtained by the two methods of attack in certain instances. The analytical work was repeated and proved. It was then discovered that the carbonate-CO₂ results,¹ as given for 2,000,000 pounds had been multiplied by that amount instead of 1,000,000. The latter figure should have been used to obtain the occurrence for the 2,000,000-pound basis, since the additions had been incorporated in only one-half of the full weight of soil. The use of the data, thus erroneously computed in the first contribution,¹ to obtain the "fixation" data given in the second paper, introduced an error in those cases where residual carbonates remained from the coarser separates. In the case of complete absorption, no error was introduced and in the case of the smaller carbonate residues the error is not of appreciable magnitude, but the magnitude of the fixation data is affected in the case of the coarser limestone and dolomite separates. Since the error was a constant factor the general conclusions are not altered, but the magnitude of the amounts of coarser limestone and dolomite absorbed by the soil is considerably changed. The single table given in each paper is therefore reprinted with complete corrections.

¹ *Soil Sci.* (1925) 20: 409.

² *Soil Sci.* (1926) 22: 111.

TABLE 1

Residual carbonates and disintegration of 2,000-pound CaO-equivalent limestone and dolomite separates from surface-zone and subsurface-zone incorporations in an acid Cumberland loam after exposure in lysimeter tanks for a period of 4 years*

| SEPARATES INCORPORATED IN SOIL | CaCO ₃ —EQUIVALENT OF DETERMINED RESIDUAL CO ₂ | | | | | RESIDUAL CaCO ₃ ± CORRECTED FOR CONTROLS | | | DISINTEGRATION OF ADDITION BY DIFFERENCE AS ACCOUNTED FOR BY ABSORPTION AND LEACHING | |
|---|---|-------------|-------------|-------------|----------------|---|-------------------------|----------------------|---|-------|
| | 1 | 2 | 3 | Average | Probable error | On basis of moisture-free soil | | On basis of addition | | |
| | | | | | | Analyses | Per 2,000,000 pounds | | | |
| | per cent | per cent | per cent | per cent | per cent | per cent | lbs. | per cent | per cent | lbs. |
| Group 1. From limestone incorporated in surface zone | | | | | | | | | | |
| 10-20 mesh..... | 0.191 | 0.192 | 0.194 | 0.192 | ±0.001 | 0.167 | 1,670 | 46.8 | 53.2 | 1,900 |
| 20-40 mesh..... | 0.051 | 0.051 | 0.053 | 0.052 | ±0.001 | 0.027 | 270 | 7.6 | 92.4 | 3,300 |
| 40-80 mesh..... | 0.028 | 0.026 | 0.033 | 0.029 | ±0.002 | 0.004 | 40 | 1.1 | 98.9 | 3,530 |
| 80-200 mesh..... | 0.031 | 0.040 | 0.034 | 0.035 | ±0.003 | 0.010 | 100 | 2.8 | 97.2 | 3,470 |
| Composite†..... | 0.094 | 0.082 | 0.094 | 0.090 | ±0.004 | 0.065 | 650 | 18.2 | 81.8 | 2,920 |
| Group 2. From limestone incorporated in subsurface zone | | | | | | | | | | |
| 10-20 mesh..... | 0.100 | 0.109 | 0.113 | 0.107 | ±0.004 | 0.086 | 860 | 24.1 | 75.9 | 2,710 |
| 20-40 mesh..... | 0.038 | 0.038 | 0.039 | 0.038 | ±0.001 | 0.017 | 170 | 4.8 | 95.2 | 3,400 |
| 40-80 mesh..... | 0.022 | 0.023 | 0.019 | 0.021 | ±0.001 | 0.000 | 000 | 0.0 | 100.0 | 3,570 |
| 80-200 mesh..... | 0.020 | 0.020 | 0.022 | 0.021 | ±0.001 | 0.000 | 000 | 0.0 | 100.0 | 3,570 |
| Composite†..... | 0.056 | 0.056 | 0.053 | 0.055 | ±0.001 | 0.034 | 340 | 9.5 | 90.5 | 3,230 |
| Group 3. From dolomite incorporated in surface zone | | | | | | | | | | |
| 10-20 mesh..... | 0.220 | 0.214 | 0.231 | 0.222 | ±0.005 | 0.197 | 1,970 | 55.2 | 44.8 | 1,600 |
| 20-40 mesh..... | 0.113 | 0.108 | 0.116 | 0.112 | ±0.002 | 0.087 | 870 | 24.4 | 75.6 | 2,700 |
| 40-80 mesh..... | 0.069 | 0.083 | 0.077 | 0.076 | ±0.004 | 0.051 | 510 | 14.3 | 85.7 | 3,060 |
| 80-200 mesh..... | 0.033 | 0.041 | 0.033 | 0.036 | ±0.003 | 0.015 | 150 | 4.2 | 95.8 | 3,420 |
| Composite†..... | 0.123 | 0.131 | 0.133 | 0.129 | ±0.003 | 0.104 | 1,040 | 29.1 | 70.9 | 2,530 |
| Group 4. From dolomite incorporated in subsurface zone | | | | | | | | | | |
| 10-20 mesh..... | 0.162 | 0.179 | 0.188 | 0.076 | ±0.008 | 0.155 | 1,550 | 43.4 | 56.6 | 2,020 |
| 20-40 mesh..... | 0.072 | 0.088 | 0.091 | 0.083 | ±0.006 | 0.062 | 620 | 17.4 | 82.6 | 2,950 |
| 40-80 mesh..... | 0.032 | 0.036 | 0.044 | 0.037 | ±0.003 | 0.016 | 160 | 4.5 | 95.5 | 3,410 |
| 80-200 mesh..... | 0.033 | 0.033 | 0.029 | 0.032 | ±0.001 | 0.011 | 110 | 3.1 | 96.9 | 3,460 |
| Composite†..... | 0.089 | 0.089 | 0.094 | 0.091 | ±0.002 | 0.070 | 700 | 19.5 | 80.5 | 2,870 |

* Constant of 2,000-pound CaO, or 3,570-pound CaCO₃ equivalences per acre.

† Equal quantities of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

TABLE 1—*Concluded*

| SEPARATES INCORPORATED IN SOIL | CaCO ₃ —EQUIVALENT OF DETERMINED RESIDUAL CO ₂ | | | | | RESIDUAL CaCO ₃ \pm CORRECTED FOR CONTROLS | | | DISINTEGRATION OF ADDITION BY DIFFERENCE AS ACCOUNTED FOR BY ABSORPTION AND LEACHING | |
|--|---|-------|-------|---------|----------------|---|-------------------------|----------------------|---|-------------|
| | 1 | 2 | 3 | Average | Probable error | On basis of moisture-free soil | | On basis of addition | | |
| | | | | | | Analyses | Per 2,000,000 pounds | | | |
| | | | | | | per cent | per cent | per cent | per cent | per cent |
| Group 5. From burnt lime controls in surface zone | | | | | | | | | | |
| CaO..... | 0.041 | 0.041 | | 0.041 | | 0.016 | 160 | 4.5 | 95.5 | 3,410 |
| CaO—MgO†..... | 0.037 | 0.041 | | 0.039 | | 0.014 | 140 | 3.9 | 96.1 | 3,430 |
| CaO—MgO§..... | 0.037 | 0.041 | | 0.039 | | 0.014 | 140 | 3.9 | 96.1 | 3,430 |
| Group 6. From burnt lime controls in subsurface zone | | | | | | | | | | |
| CaO..... | 0.038 | 0.031 | | 0.035 | | 0.014 | 140 | 3.9 | 96.1 | 3,430 |
| CaO—MgO†..... | 0.041 | 0.041 | | 0.041 | | 0.020 | 200 | 5.6 | 94.4 | 3,310 |
| CaO—MgO§..... | 0.041 | 0.041 | | 0.041 | | 0.020 | 200 | 5.6 | 94.4 | 3,310 |
| Group 7. From untreated controls | | | | | | | | | | |
| Surface..... | 0.026 | 0.026 | 0.024 | 0.025 | ± 0.001 | | | | | |
| Subsurface..... | 0.020 | 0.019 | 0.025 | 0.021 | ± 0.002 | | | | | |

† Burnt dolomite.

§ Mixture of separately calcined oxides.

TABLE 1
Fixation of Ca-Mg from a 3570-pound CaCO₃-equivalence (2000 pounds CaO) of Ca(OH)₂, CaO-MgO, and limestone and dolomite separates in surface-zone and subsurface-zone incorporations with a loam soil under outdoor conditions for a period of 4 years
 Results are given in terms of CaCO₃-equivalence per 2,000,000 pounds of soil, moisture-free basis

| TREATMENT | SURFACE-ZONE INCORPORATION | | | | | SUBSURFACE-ZONE INCORPORATION | | | | | FIXATION FROM SURFACE-ZONE INCORPORATION OVER THAT FROM SUBSURFACE INCORPORATION | |
|---------------------------|----------------------------|---|---|--|----------|-------------------------------|---|---|--------|----------|--|----------|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) |
| | pounds | Total CaCO ₃ equivalence accounted for by both fixation and leaching | Total Ca-Mg outgo in excess of that from controls | Fixation—Full depth of soil considered | per cent | pounds | Total CaCO ₃ equivalence accounted for by both fixation and leaching | Total Ca-Mg outgo in excess of that from controls | pounds | per cent | pounds | per cent |
| Ca(OH) ₂ | 160 | 3,410* | 271 | 3,139 | 87.9 | 140 | 3,430 | 1,148 | 2,282 | 63.9 | 857 | 24.0 |
| CaO-MgO†..... | 140 | 3,430 | 231 | 3,199 | 89.6 | 200 | 3,370 | 1,215 | 2,155 | 60.4 | 1,044 | 29.2 |
| CaO-MgO‡..... | 140 | 3,430 | 156 | 3,274 | 91.7 | 200 | 3,370 | 1,090 | 2,280 | 63.9 | 994 | 27.8 |
| L.S. 10-20..... | 1,670 | 1,900 | 78 | 1,822 | 51.0 | 860 | 2,710 | 506 | 2,204 | 61.7 | —382 | -10.7 |
| L.S. 20-40..... | 270 | 3,300 | 170 | 3,130 | 87.7 | 170 | 3,400 | 817 | 2,583 | 72.3 | 547 | 15.4 |
| L.S. 40-80..... | 40 | 3,530 | 211 | 3,319 | 93.0 | 000 | 3,570 | 1,098 | 2,472 | 69.2 | 847 | 23.8 |
| L.S. 80-200..... | 100 | 3,470 | 226 | 3,244 | 90.9 | 000 | 3,570 | 1,133 | 2,437 | 68.3 | 807 | 22.6 |
| L.S. Comp.§..... | 650 | 2,920 | 51 | 2,869 | 80.4 | 340 | 3,230 | 900 | 2,330 | 65.3 | 539 | 15.1 |
| Dol. 10-20..... | 1,970 | 1,600 | 38 | 1,562 | 43.8 | 1,550 | 2,020 | 153 | 1,867 | 52.3 | —305 | -8.5 |
| Dol. 20-40..... | 870 | 2,700 | 105 | 2,595 | 72.7 | 620 | 2,950 | 486 | 2,464 | 69.0 | 131 | 3.9 |
| Dol. 40-80..... | 510 | 3,060 | 152 | 2,908 | 81.5 | 160 | 3,410 | 805 | 2,605 | 73.0 | 303 | 8.5 |
| Dol. 80-200..... | 150 | 3,420 | 207 | 3,213 | 90.0 | 110 | 3,460 | 1,013 | 2,447 | 68.5 | 766 | 21.5 |
| Dol. Comp.§..... | 1,040 | 2,530 | 115 | 2,415 | 67.6 | 700 | 2,870 | 689 | 2,181 | 61.1 | 234 | 6.5 |

* Assumption of theoretical carbonation.

† Calcined dolomite.

‡ Corresponding mixture of separately calcined CaO and MgO.

§ Equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

¶ Basis of CaCO₃-equivalence of addition.

THE USE OF BACTERIOSTATIC DYES IN THE ISOLATION OF RHIZOBIUM LEGUMINOSARUM FRANK

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The place of dyes for the differentiating of various groups of bacteria has been studied quite extensively in recent years and various applications have been made of the new facts. An example of this is the use of eosin-methylene blue agar plates for the differentiation of *Escherichia coli* from its close relative, *Aerobacter aerogenes*. Another recent development in the use of the bacteriostatic effect of dyes upon certain groups of bacteria is for the purpose of ridding mixed cultures of undesired forms. This is done in the presence of nutrient media, so that the organism unaffected by the dye will outgrow the undesired forms. The tri-phenyl-methane group of dyes has been especially studied in this connection, and of these, crystal violet probably has received the most attention.

PREVIOUS STUDIES

Hall and Ellefson (5) found in the presumptive test for *Escherichia coli* that a concentration of $1:100,000$ of crystal violet in the culture medium would prevent the development of interfering anaerobes, while not inhibiting the growth of *Esch. coli*. In fact all gram-positive organisms were found to be less resistant to the action of the dye than were gram-negative organisms.

Skinner and Murray (7) found that the addition of crystal violet in a concentration of $1:100,000$ in the standard eosin methylene blue agar (American Public Health Association) inhibits the development of spreading colonies and in no way makes more difficult the identification or isolation of colon bacilli. On the contrary, the colonies of the colon bacilli were found to be even more typical than those on standard agar. Grossley (4) had previously shown that there was a direct correlation between gram-positiveness and the inhibition of growth by gentian violet, which is merely crystal violet with dextrin as an impurity. That is to say, gentian violet allowed gram-negative organisms to grow while it actually killed gram-positive organisms in the same concentrations.

Churchman (1) found that gram-negative organisms differ in their resistance to the bacteriostatic effect of crystal violet. As an example, he found that

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Eberthella typhi (Schröter) Buchanan is more resistant to crystal violet than *Esch. coli* (Escherich) Castellani and Chambers.

Vandecaveye (8), in a study of the gram-negative root nodule bacterium, *Rhizobium leguminosarum* (Frank), found that this organism was more resistant to the bacteriostatic action of the tri-phenyl-methane dyes than *B. radiobacter*, a common contaminant of cultures of *R. leguminosarum*.

PRESENT STUDIES

The common method of isolating *Rhizobium leguminosarum* is so tedious and uncertain in the hands of the inexperienced that a shorter and more efficient method is much to be desired. This is especially true in view of the difficulty of differentiating it from the common contaminant, *B. radiobacter*, which is very similar to *R. leguminosarum* in cultural and morphological characteristics. The differential resistance of *R. leguminosarum* and *B. radiobacter* to the action of the tri-phenyl-methane dyes offers a distinct possibility that these dyes and dyes belonging to other chemical groups may be of assistance in simplifying the method of isolation of the root nodule organism.

Dyes may be used for the isolation of an organism in several ways. One of these is to suspend the bacteria (mixed culture) in an aqueous solution of the dye for a period of time sufficient to kill the contaminating species and then transfer the survivors to a culture medium suitable for the growth of the desired species. Another method is to seed a mixed culture into a medium containing such a concentration of the dye that the growth of the undesired species will be inhibited while the desired species will grow sufficiently to produce visible colonies. The ideal dye for the isolation of legume root nodule bacteria would be one which could kill or retard in growth all of the organisms in the nodule except the desired one, *R. leguminosarum*. The following experiments were carried out with this in mind.

From the results of the investigations mentioned under "Previous Studies" it would seem that success would be more likely to result from the use of dyes of the tri-phenyl-methane group. However, only dyes which are water soluble could be used for the present studies. As classified by Conn (2) the following members of this group were used:

Basic Dyes: crystal violet, rosaniline hydrochloride, malachite green, basic fuchsin, methyl green, brilliant green, and methyl violet.

Acidic Dyes: acid fuchsin, acid green G, and methyl blue.

A few dyes belonging to other chemical groups also were used in a few experiments. These were as follows:

Quinone-Imide Group: methylene blue and safranin. These are basic dyes.

Xanthene Group: eosin B, eosin Y, and rose bengal. These are all acidic dyes.

Azo Group: congo red.

All of the experiments reviewed here were carried on with crushed nodules or freshly isolated but badly contaminated cultures of alfalfa and bean strains of local origin. Old cultures were not used because preliminary studies had shown that organisms within the nodule are more resistant to dyes than are their descendants after the culture has been subjected for some time to growth under artificial conditions. A plausible explanation of this fact (not verified by microscopic studies) is based on the hypothesis that the organisms within the nodule are equipped with a heavier and thicker coating of slime than those of cultures grown artificially for some time. Fred (3) has shown that there is a correlation between the amount of slime production and the resistance of the organism to crystal violet. He also has shown that the alfalfa-sweet clover group is more susceptible to hydrogen-ion concentration than other physiological groups. This physiological group also is more susceptible to the bacteriostatic action of dyes than the other groups.

The alfalfa and bean physiological groups were chosen because they represent the extremes in resistance to hydrogen-ion concentration. According to Fred (3) the alfalfa and sweet clover cross inoculation group is inhibited in growth at $\text{pH} = 4.9$ and the bean group at $\text{pH} = 4.2$.

Several methods of applying the bacteriostatic action of the dye were studied with the idea of finding the one best suited for obtaining a pure culture.

As a check on the different methods used, the cultures obtained by the use of dyes were examined microscopically for evidence of admixture of the desired organisms with others which differ noticeably from them in morphology. To test for the absence of *B. radiobacter*, which has the same microscopic appearance as *R. leguminosarum*, the cultures which gave evidence of purity under the microscope were tested by the milk and potato tests of Löhnis and Hansen (6). These tests consist of inoculating these media with the organism to be tested and incubating for two weeks. In the case of the potato slant cultures, if the culture is a pure one of *R. leguminosarum*, the slant will have a thin slimy growth containing no pigmentation. If *B. radiobacter* is present a distinct brown coloration is produced. In the case of the milk cultures, *R. leguminosarum* produces a thin distinct serum layer on the top of the milk which is of a slimy nature and leaves the remainder of the milk unchanged in any way. In the event that *B. radiobacter* is present the milk will have the distinct serum layer on the surface, as in the case of *R. leguminosarum*, but in addition the milk portion below the serum layer will have a brown coloration.

As a check on the effect the dyes might have on the inoculating efficiency of the organisms which were isolated by this method, some of the cultures that were found to be pure were used to inoculate the species of plant from which they were obtained originally. This was done to discover the degree of nodule production they may have retained after the dye treatment.

DETAILED METHODS AND RESULTS

Technique Number One was one in which a dye agar was used. The detailed technique, in brief, was to make up relatively large amounts of the desired concentration of dye agar by adding a calculated amount of an aqueous stock solution of the dye to a flask of Ashby's agar. This was then sterilized in the autoclave at 10 pounds for 20 minutes. When the agar was removed from the sterilizer, plates were poured at once and allowed to solidify. A modification of this procedure was to add the dye to the previously sterilized agar and then pour the plates into petri dishes to allow the agar to solidify. After the agar had become solid the cultures were streaked heavily upon the surface. This technique did very little more than hold down the growth of any spreading organisms that might be present in the cultures, and even in this it was not always effective.

Technique Number Two was one making use of solutions of the desired dyes in Ashby's solution. The detailed procedure in this method was to make up the dye dilutions, with Ashby's solution, in small flasks, sterilize, and after sufficient cooling inoculate the desired cultures into the aqueous solutions of the dye. They were incubated for a week before a check was made as to their purity. The dilutions of the dye varied from 1:100 to 1:100,000 depending upon the dyes used. By removing a loopful of the suspension of the growing organisms from the flask and inoculating Ashby agar slants the purity of the resulting growth was determined. The results of this method were not very encouraging as it was found that very few of the dyes remained stable throughout the time of incubation. Some of the dyes decomposed very rapidly and were of no value whatever in their selective action on undesirable microorganisms. The dyes which remained relatively stable in this method also showed insufficient selective action.

Technique Number Three was one in which the probable desirable germicidal action of the different dyes was looked into. In this method 1, 0.75, 0.5, and 0.25 per cent solutions were made of the dyes to be studied. To $2\frac{1}{2}$ cc. of the dye solution in a test tube, three to five drops was added of a reasonably heavy suspension of organisms from which the desired organism was to be isolated. From this dye-organism mixture a loopful of suspension was taken after varying lengths of time; that is, a loopful of suspension was removed at the end of $\frac{1}{2}$, 1, 2, 3, 4, 5, 7, 10, $12\frac{1}{2}$, 15, 20, 30, 45 minutes, and 1 hour. A check consisting of a suspension of the organisms in sterile water was treated in the same fashion and a suspension removed at the same periods of time. This check was for the purpose of showing that any results obtained were not due to mere dilution. The loopfuls of suspension, removed at the varying intervals of time, were streaked out upon Ashby agar plates. After a week's incubation, microscopic observations for purity were made on the resulting growth, methylene blue being used to stain the smears.

The results of technique number three were considerably more encouraging than those of the preceding methods. Insufficient success was obtained to

class this method as one efficient enough for practical use. By this method it was found that those dyes which have indications of possible favorable selective action are crystal violet, brilliant green, and malachite green. Those dyes which gave some indication of selective action but did not have this characteristic strong enough to warrant their use in a more extensive study were methyl green, methyl violet, basic fuchsin, methylene blue, eosin B, eosin Y, rose bengal, and safranin. Acid fuchsin was very undesirable in its action since it actually killed the legume organisms.

In the use of this technique it was noted that considerable trouble was sometimes experienced when spores of some of the undesired organisms were present, as they withstood the germicidal action as long as the legume root nodule organisms and produced a contaminated culture. It was noted also that it was very difficult to make use of the selective action of crystal violet by this technic, for its action is very rapid in killing both the undesired and the desired organisms. Trouble arose in spacing the periods of time close enough together to check up on the action and in making dilutions which would be efficient without killing everything. In the short time (10 minutes) that the living organisms remained in contact with the crystal violet the action was noted to be decidedly favorable in that nothing but the slime-producing organisms of the root nodule remained. Brilliant green and malachite green were more controlled in their action in that they were slower in their selection of any of the organisms. The only indication of their action here was that it tended distinctly in the desired direction; that is, the legume root nodule bacteria were allowed to live longer than any "spreader"-forming organisms. No tests were made in this method on potato or milk for the presence of *B. radiobacter* because this technique was superseded by the following one.

Technique Number Four made use of the bacteriostatic nature of the dyes which were tested by this method. This characteristic of the dyes, studied in connection with the organisms in question, was found to be the best for successful isolation of the legume root nodule bacteria.

The detailed procedure of this technique was to inoculate petri dishes with a water suspension of the contents of legume root nodules and to pour over these inoculated dishes dye agar of the desired dye concentration after being cooled to a degree not uncomfortably warm to the eyelid.

The nodule suspension was obtained by the following procedure: Fair-sized nodules were selected from the plants, cut with a small pair of scissors from as much of the root as was practicable, washed as clean as possible from adhering soil, and crushed with a stirring rod in a tube of tap water. In this experiment a number of nodules were crushed in a little water in a large tube and diluted with tap water until the water was faintly turbid. The petri dishes were inoculated with a few drops of the turbid liquid by means of a pipette. The suspension was kept for some time, as it was found that the organisms remained alive in a watery suspension for a period of at least six months.

In making the dye agar, large glass test tubes were filled with enough Ash-

by's agar to make the volume after sterilization very close to 25 cc. After the agar was sterilized it either was used before it had a chance to solidify or was allowed to solidify and then stored for a few days. In the case of its immediate use the agar was cooled to a point not quite cool enough to bear on the cheek and treated with the desired dye. In case the agar had been allowed to solidify it was melted and then cooled down in the manner just described. The dye treatment consisted of adding enough of a 1 per cent aqueous-alcoholic solution of the dye to bring the concentration to the desired point. Just enough alcohol was added to the stock solution of the dye to insure complete solution and to prevent decomposition. In many cases it was found that the dyes were not very stable in water so that the alcohol was found to be very essential.

After the dye had been added to the agar and solution accomplished the agar was cooled to a point where the test tube could be comfortably held to the eyelid or cheek. When at that point the agar was poured, as has already been stated, into the previously inoculated petri dishes and allowed to solidify. After solidification the dishes were incubated at room temperature for a week and the results recorded.

The dyes which were tested by this method were crystal violet, malachite green, rosaniline hydrochloride, safranin, eosin Y, and rose bengal.

Safranin, eosin Y, and rose bengal were run with the following dilutions of dye in Ashby's Agar: 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800, and 1:25600. Above 1:100 there was little bacteriostatic effect on bacterial species and none of the concentrations used restrained the growth of molds. On the whole these dyes were too weak to be useful and they were eliminated after one trial.

Rosaniline hydrochloride was run at the following dilutions: 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200. This dye also allowed the growth of molds to too great an extent for the satisfactory isolation of *R. leguminosarum*. At the 1:100 dilution of the dye the alfalfa strains showed no growth and the bean strains a few colonies of a nature characteristic of the legume root-inoculating bacteria. This dilution failed to show any tendency to retard the growth of molds since the dye medium had as heavy a crop of mold growth as did the check plates without dye. The mold which appeared was not positively identified but was either *Rhizopus* or *Mucor* and proved very bothersome in making the final isolation of colonies in a pure condition. The bacterial colonies which developed in the bean nodule plates in this dilution (1:100) were all pure and appeared to be *R. leguminosarum*, as judged by observing the colonies with the naked eye. At 1:200, colonies put in their appearance on the alfalfa nodule plates and the number on the bean nodule plates increased accordingly. The 1:400 plates gave much the same results as did the 1:200, and in the 1:800 and up to 1:3200 other bacterial growths began to appear. Under the microscope the cultures obtained from the 1:100, 1:200, and 1:400 plates all upheld the evidence pointed out by the macroscopic observations of the plates;

that is, colonies of the legume root-inoculating organisms could be obtained in absolute purity. No tests were made from the cultures obtained by the use of this dye for *B. radiobacter*, because molds were so troublesome when this dye was used. For this reason the dye was considered insufficient for our purpose.

Malachite green gave considerably more encouraging results than rosaniline hydrochloride in its selective reactions. With this dye, in the first series of tests, dilutions of 1:100, 1:200, 1:300, 1:400, 1:500, 1:600, 1:700, 1:800, and 1:900 were made and it was found that no dependable growth of any sort would be allowed by this dye at any dilution from 1:100 to 1:400, for no growth whatever was observed up to that point in the alfalfa nodule cultures and only a few colonies on the 1:500 plates in the case of the bean nodule culture (probably due to improper mixing of the dye at that particular dilution). From 1:600 on, the number of colonies of *R. leguminosarum*-like nature increased and continued to be the only organisms on the dye plates up to a dilution of 1:800. Molds were inhibited by this dye. On the plates with dye dilutions of 1:600 and up, the purity of the colonies was a matter of some question. All secondary experiments with this dye, after the first series was made, were run with dilutions ranging from 1:600 up to 1:800. A dilution of 1:600 or 1:700 was found to allow the growth of a fair representation of the desired type of colonies, which under the microscope were found to be pure cultures of what appeared to be the desired organisms, and yet prevented the growth of any other types of bacteria. Cultures of the colonies on the two types of plates—alfalfa and bean—were saved from this dye and run for purity on milk and potato, as has been mentioned before, and the purity was found to be rather inconsistent and undependable at any concentration.

The dye which gave the most decided and favorable results was crystal violet. Dilutions of 1:100, 1:200, 1:300, 1:400, 1:500, 1:600, and 1:700 were made for the first experiment with this dye and dilutions of 1:200, 1:300, 1:400, 1:500 and 1:600 for all the subsequent experiments. With this dye, as in the others, it was found that the alfalfa strain showed a slightly weaker tendency to recover from the effects of the dye than did the bean strain, for at the 1:200 dilution the bean strain gave several medium-sized colonies, whereas the alfalfa strain had but one colony. With the 1:300 dilution the alfalfa strain colonies began to show up, and at a dilution of 1:400 one could depend on a fair representation of colonies of the organisms having the characteristics of *R. leguminosarum*. Dilutions of 1:500 and 1:600 were thought to be the most satisfactory, as at these dilutions colonies in sufficient numbers and of sufficient size were obtained in every test and yet the concentration was such as to prevent the appearance of colonies of any other type. At 1:500 a few colonies of gram-negative organisms of other types were noted. No molds appeared on any of the plates in which this dye was used; this proved of some distinct advantage in the picking of the colonies.

Cultures were obtained from colonies which grew on the plates with the dilutions of 1:500, 1:600, 1:700, 1:800, 1:900, and 1:1000 of crystal violet. These

cultures were all tested by the method of Löhnis and Hansen (6) for freedom from *B. radiobacter*, as already described in this paper. They were also tested for inoculating efficiency, as has already been stated. The purity test on potato and milk showed all the cultures obtained by this dye up to and including those obtained from a dilution of 10^{-7} to be free from *B. radiobacter*. The 10^{-8} dilution culture and those from higher dilutions gave very irregular results in the matter of freedom from *B. radiobacter*, so that it would appear that in these dilutions this contaminant was not eliminated.

The cultures obtained from the crystal violet plates with dye dilutions of 10^{-7} and 10^{-8} for both the alfalfa and bean strains were tested for their inoculating efficiency by either growing the plants in question in a large test tube of Ashby's agar or in sterile sand inoculated with the strain of the organism isolated by the dye method. In either event the plants were allowed to grow for five or six weeks in the presence of the organisms and then observed for nodules. In the case of the alfalfa strain isolated from the 10^{-7} dye dilution, the test tube method was used; all other tests were made in sterile sand which had been inoculated with the strain of the organism to be tested. In the tube of Ashby's agar, in which the alfalfa plant was grown in company with the culture isolated from the 10^{-7} dye agar plate, a heavy crop of nodules was noted, showing that at that concentration the efficiency of the strain was not in any way weakened. Moreover, on all the other tests for efficiency of the strains isolated through the use of crystal violet, nodules were formed in very satisfactory amounts, which gives further evidence that the organisms survive the severe treatment to which they are subjected during the contact with the dye.

A presumably pure culture of *B. radiobacter* was treated by the crystal violet method of isolating the legume-inoculating bacteria and colonies of a characteristic nature were obtained in dilutions, even below 10^{-7} . These strains were then tested for purity by the means already described, and it was found that *B. radiobacter* was absent. They were at the same time used to inoculate a number of alfalfa and bean plants and found to induce a good crop of nodules of the alfalfa roots. This was undoubtedly due to the culture of *B. radiobacter* being mixed with alfalfa-inoculating bacteria. Before subjecting it to the crystal violet treatment the culture was inoculated into milk and found to give the brown coloration, showing that *B. radiobacter* was present.

As a final test of the practicability and simplicity of this method of isolating *R. leguminosarum* by the aid of dyes, an exercise was made up for a class in beginning bacteriology and submitted to them for trial. The class was uniformly successful and obtained pure cultures of *R. leguminosarum* at the very first trial. The dilutions suggested for the use of the class were 10^{-7} and 10^{-8} . A few were asked, as a demonstration of the extremes of the original experiment, to run dilutions of 10^{-9} and 10^{-10} . The findings in the "side" experiments were similar to those obtained by the writer. No tests for purity were made by the method of potato or milk inoculations on the cultures obtained by the class.

CONCLUSIONS

From the foregoing results, which were repeated several times, it is reasonable to conclude:

1. That subjecting *R. leguminosarum* to severe treatment with dyes does not in any way affect the ability of the legume root nodule-producing bacteria to produce nodules on the species of plants from which they were originally isolated. This was arrived at by observing that cultures recovered from dye plates which almost entirely inhibited all growth were still able to produce nodules on the plants.

2. That such a method of isolating *R. leguminosarum* is possible and practicable through the use of crystal violet dissolved in Ashby's agar in concentrations of 1 part of dye to 10,000 of medium, down to 1 part of dye to 15,000 parts of medium. This method was found so simple and convenient that beginning students in bacteriology could get successful results in their first trials.

3. Other dyes which gave promising results, although not so clear-cut as the results with crystal violet, were rosaniline hydrochloride and malachite green.

4. The foregoing results were confined to nodules from locally grown alfalfa and bean plants. Time did not permit of extending these studies to the nodules of other leguminous plants nor to plants from other parts of the country.

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CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: V. THE RÔLE OF MICROÖRGANISMS IN PEAT FORMATION AND DECOMPOSITION¹

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Microörganisms in general play four distinct parts in the chemical transformations leading to peat formation and decomposition:

1. Those microörganisms which are active during the first stages of decomposition of the plant residues, either before or after the residues have become submerged with water; in these processes of disintegration some of the chemical constituents of the plants rapidly disappear. Certain groups of organisms, largely fungi on the surface of the bog and bacteria below the surface of the anaerobic medium, bring about the decomposition of the sugars, of certain hemicelluloses, of the celluloses, and of some proteins and their derivatives. The carbon dioxide and ammonia thereby liberated are used immediately by the growing plants; this process is of great importance in bogs poor in nutrients, like the highmoor peats.

2. Microörganisms active in the various horizons of the peat profile, long after the initial stages of decomposition have passed. Here we are dealing almost entirely with facultative and obligate anaerobic bacteria. The pockets of gas rich in hydrogen and methane, as well as certain putrefactive odors, frequently found at various depths of the peat profile are due to the gradual decomposition of the celluloses, of the proteins, and of other complexes by these bacteria.

3. Microörganisms active in the decomposition of the organic complexes in the peat, after the bog is drained, in the case of lowmoor and sedimentary peats, or drained and limed, in the case of the highmoor peats. Here we are dealing with various fungi, aerobic bacteria, and actinomyces decomposing the resistant peat complexes with the liberation of large quantities of ammonia, which is rapidly changed to nitrates by nitrifying bacteria. These nitrates may accumulate in quite considerable quantities in the surface layers of the drained peat. Frequently and under certain conditions, especially in highmoor bogs receiving excessive amounts of lime, nitrate reducing bacteria may become active; this leads to losses of nitrogen to the atmosphere.

4. Microörganisms that have contributed directly by their cell substance to the formation of certain peats. This is true especially of sedimentary or allochthonous peats in which fungus spores and mycelium, as well as various algae and bacteria, may be quite abundant.

Peat is formed because the saturation of the bog with water produces anaerobic conditions. This does not prevent the growth of plants adapted to that environment, but it does prevent the growth of fungi, actinomyces, and aerobic bacteria which would be capable of decomposing the plant residues. The obligate and facultative anaerobic bacteria favored by these conditions are capable of attacking only some of the organic complexes, leaving the other

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constituents to accumulate, and thus give origin to peat. The lignins, either as such or in a modified form, certain nitrogenous substances (proteins, etc.), and various hemicelluloses predominate in lowmoor peats; whereas waxes, certain celluloses and hemicelluloses, and lignin-like complexes predominate in the highmoor peat formations.

No other phase of peat investigation has been more confused and no other phase suffers from a greater lack of accurate information than the proper understanding of the rôle played by microorganisms in peat formation and in peat transformation. Some chemists deny altogether the fact that microbes take any part in the transformation of plant substances into peat; they either consider the process as one of spontaneous decomposition of plant material, giving origin to peat, or believe that the transformations are a result of the action of atmospheric agencies, as those of oxidation and reduction.

Potonié (29), for example, considers the process of peat formation as one of "self decomposition," which represents the last phase of oxidation, decomposition, and fermentation processes. According to Früh (13), peat formation is not a result of bacterial action, but consists in a slow breakdown, at low temperatures and in the absence of oxygen, of the plant constituents, with an inner oxidation, resulting in the liberation of water:



He states quite specifically that the process of peat formation is not one of fermentation nor is it one of bacterial action, but consists in a slow decomposition of plants in the complete absence of oxygen, due to the presence of water and at low temperatures, but, he adds, "Spaltpilze haben mit der Torfbildung nichts zu tun."

Similar ideas have been expressed by Kauko (20) and others. Oden (27) also speaks of the rôle of atmospheric agencies in peat formation. Some of the chemists consider the whole problem of peat formation in even simpler terms: according to these conceptions, the carbohydrates which are formed from the decomposition of the celluloses and the amino acids produced from the proteins combine at high temperatures to give dark colored substances (25). The fact that neither sugars are formed nor do amino acids accumulate under the natural soil and bog conditions, as well as the fact that the required high temperature will not be attained under such conditions, do not matter much, as long as the logic is correct.

Kürschner (22) is, therefore, quite justified in speaking that all the processes termed "self-decomposition," "breakdown," "autolysis," etc. are merely attempts to explain x by y , or one unknown factor by another equally unknown without any attempt to get a deeper insight into the problem.

An understanding of the functions of microorganisms in peat formation necessitates a knowledge of the nature of the organisms commonly found in the various horizons in the natural peat profile, their activities, and their rôle in peat transformation. An attempt will be made in this paper to sum-

marize our present information and to throw further light upon this complicated problem.

HISTORICAL

As mentioned above, Fröh (13) was among the very first to report in 1883 that peat bogs are free from bacteria, i.e. are practically sterile. However, the bacteriologist Gaffky (14) claimed that bacteria are present in peat bogs. The well-known peat investigator, Weber, stated in 1890 that bacteria are found only in the surface layers of peat. Both Proskauer (30) and Benni (5) soon demonstrated that bacteria may be found even in the lower depths of peat bogs.

Ramann and associates (31) found bacteria in great abundance in the upper layers (0 to 5 cm. depth) of young peat formations, the numbers ranging from 200,000 to 2,710,000 cells of bacteria per gram of dry material; the existence of these organisms at lower depths was doubted, however. Stålström (36), as well, found that all peat samples taken from a depth of 50 cm. and lower were sterile; he claimed, therefore, that the presence of microorganisms is limited to the upper 20 cm. of the bog.

Fabricius and von Feilitzen (10) reported the presence of 56,000 to 225,000 bacteria per gram of moist peat taken at a depth of 35 cm. Fischer (11) found as many as 1,000,000 bacteria in one gram of well decomposed peat and 950,000 in young peat. Ritter (32) found fewer bacteria in young than in old peat; he rarely encountered peat entirely free from bacteria. Even assuming the existence of bacteria in peat bogs, Wehmer (45) still doubted whether the activities of these organisms could fully explain the problem of "humin formation."

All the aforementioned investigators used the aerobic plate method (with meat peptone agar or gelatin, or peat extract agar) for determining the abundance of bacteria in peat. Their results point to the existence in natural peat of very small numbers of bacteria capable of developing under these conditions. These numbers were at best only about one-tenth of the numbers of bacteria commonly found in field soils by the use of the same method. It is doubtful whether the methods used for determining the presence and abundance of microorganisms in peat were satisfactory in most of the aforesaid studies. Keppeler (21) has already expressed his grave doubts of the accuracy of those results, because of difficulties involved in experiments of this nature.

Begak (4) recently made a study of the distribution of bacteria in various profiles of a sphagnum peat, using meat-peptone agar as a nutrient medium. The numbers were found to be around 4,000 cells per gram of peat in the upper 10 cm. of the bog, increasing to 90,300 at 25 to 30 cm., then diminishing with depth, so that, at 2.0-2.5 meters depth (at the Grenzhorizont), there were only 2,800 cells per gram; below that layer, the numbers increased again to 9,400 cells per gram of peat. The numbers of bacteria in the waters of the dead sphagnum and in the sphagnum itself were constant throughout the summer,

namely, about 4,000 cells per gram. About eight times as many bacteria were found in the well-decomposed layers of the peat as in those that were only partly decomposed. It is interesting to note that the abundance of organisms found by Begak, by the use of the plate method, in the Grenzhorizont was later confirmed by microscopic examinations; the author noted thereby also a very abundant flora of fungi forming very minute colonies.

By the use of the direct microscopic method of soil examination (after Winogradsky), Begak found 323 to 715 millions of bacteria per gram of moist sphagnum, which would give three to seven billion cells in each gram of dry sphagnum. These bacteria were made up of 1.25 per cent large rods, 60.0 per cent short rods, 20 per cent cocci, and about 20 per cent thick rods and small cocci. This abundant microflora of bacteria as well as an abundant development of fungi were believed to prove that these are the active agents in the decomposition processes taking place in the peat. The acidity of the sphagnum was equivalent to pH 3.3 to 4.2. The bacteria present there were found to be adapted to this high acidity.

Ritter (32) previously criticized the Koch plate method as altogether unsuitable for giving any idea concerning the abundance of the bacterial population in peats. The nitrifying, anaerobic, nitrogen-fixing, and other organisms do not develop at all on this medium. The colloidal nature of the peat makes an even distribution of the bacteria even more difficult. His results in general showed that the cell content of uncultivated highmoor peats is usually very low; young or little decomposed peat is always poorer in bacterial cells than well-decomposed peat. Peat cultivated for a long time, limed and fertilized, was found to contain incomparably more bacteria than untreated peat; when peat is well decomposed, it may contain more bacteria than mineral soils. An untreated peat receiving only an application of lime showed a relatively moderate increase in the bacterial cells. Lowmoor peats contained in all cases more bacteria than highmoor peats, even in an uncultivated state. As in mineral soils, the numbers of bacteria were found to decrease rapidly with depth, but they were still present even at a depth of 50 cm., when infection was fully excluded. A difference in the composition of the medium could not show any great variation in the numbers of bacteria. It may be of interest to call attention, in this connection, also to the results of White and Thiessen (46), who reported the presence of anaerobic bacteria in peat bogs even at a depth of 9m.

The nature of the peat was found to exert a marked influence upon the nature and abundance of the bacterial population. Stalström (36) found more bacteria in lowmoor peats than in highmoor peats; drainage brought about a great increase in bacterial development, especially when the peat is mixed with clay or manure, Fabricius and von Feilitzen (10) explained the low bacterial content of highmoor peats by the high acidity; drainage alone did not bring about any marked bacterial multiplication; liming, cultivation, manuring, and treatment with sand brought about considerable development

of bacteria. Well-manured and cultivated highmoor peat soil, however, contained as many bacteria as lowmoor peat soil under the same conditions. Soil temperature was also found to influence appreciably bacterial development. These results can be summarized as follows:

| | <i>Numbers of bacteria per gram, by the plate method</i> |
|---|--|
| Raw, uncultivated peat..... | 138,500 |
| Drained, but not cultivated peat..... | 200,300 |
| Freshly cultivated, highmoor peat treated with sand and lime..... | 6,900,400 |
| Long cultivated highmoor peat treated with sand, lime, and manure.. | 6,224,500 |
| Same, under fallow..... | 7,801,000 |
| Same, with oats grown..... | 7,175,000 |

Arnd (1) also found that the addition of lime to highmoor peat greatly stimulates the development of various groups of bacteria, especially the ammonia-forming organisms, although these are present abundantly in natural peat bogs of different types; the numbers and activities of these organisms in the deeper layers are considerably less than in the upper layers. Drainage, cultivation, and liming of highmoor peats were all found (34) to have a marked effect in increasing the numbers and activities of various groups of microorganisms, largely in the surface layers of peat.

It has been shown repeatedly that nitrifying bacteria are absent in highmoor peat bogs, although Chouard (7) observed that nitrification takes place even in certain acid peats. When a highmoor peat is moderately limed, no nitrification will take place within the first year; an excessive addition of lime will bring about rapid development of nitrifying bacteria. A moderate amount of lime will increase crop productivity, but an excess of lime above certain limits will not result in such high crop yields. This was explained (23) by the fact that the increase in the amount of lime added to the peat stimulates the development of denitrifying bacteria, organisms which may bring about considerable losses of nitrogen (16).

Asotobacter is absent in highmoor peat, while *Bac. amylobacter* is present both in cultivated and in uncultivated peat. Leguminous plants are totally absent in highmoor peats. In lowmoor peats nitrite and nitrate forming bacteria are active. Cellulose decomposing bacteria are abundant in the lowmoor peats, especially when soluble P_2O_5 is added. For the development of these bacteria in highmoor peats, the addition of calcium carbonate is essential.

According to Tacke (38), the addition of stable manure to peat has a favorable effect in stimulating the liberation of nutrients. The activities of the microorganisms were measured by adding 500 mgm. of nitrogen in the form of peat to 500 gm. of sandy soil; this was adjusted to optimum (16 per cent) moisture content and incubated for 70 days at 22°. Manured peat liberated 20.5-22.2 mgm. of nitrogen as nitrate, whereas unmanured peat liberated only 6.3 mgm. of nitrate nitrogen. The favorable action of manure was ascribed to the stimulating effect upon the activities of microorganisms in the peat.

To bring a highmoor peat into a condition when it can grow agricultural crops profitably, it has to be first drained, then fertilized with phosphorus, potassium, and, in the absence of leguminous plants, with nitrogen, and finally limed. These treatments modify considerably the activities of microorganisms, both qualitatively and quantitatively. Drainage permits the aeration of the peat material, penetration of oxygen thus enabling the aerobic organisms to develop; it produces a change from an anaerobic to an aerobic system. The removal of the water allows the soil to become warmed, thus influencing favorably the activities of microorganisms. Liming of a highmoor soil creates favorable conditions for the activities of many organisms. Arnd believed that even fungi may thus be favorably affected.

Although the Remy solution method was used by Arnd (1) for comparing the activities of microorganisms in different layers of a highmoor peat under different treatments, a method not very satisfactory for measuring the activities of the microbial population in normal field soils, marked differences were obtained: surface material from an untreated peat bog produced, in six days, from a 50-cc. peptone solution, 3.1 mgm. of nitrogen as ammonia, while peat from a depth of 20-40 cm. produced only 0.8 mgm. nitrogen as ammonia. The same peat, when drained, limed, and fertilized produced 8.2-10.3 mgm. of ammonia nitrogen; when drained, limed, fertilized, and manured peat was used, 27.8 mgm. of ammonia nitrogen was liberated.

In general, highmoor peats were found to be considerably less active biologically than lowmoor peats. Christensen (6) characterized these two types of peat microbiologically as follows:

Highmoor peats possess a low peptone-decomposing capacity, no nitrifying power, considerable denitrifying capacity, and a very limited microbial population capable of decomposing celluloses and mannitol. Lowmoor peats have a strong peptone-decomposing, nitrifying, denitrifying, and mannitol-decomposing capacity but a low cellulose-decomposing power. In the latter respect different peats may vary considerably.

Lowmoor peats were found to contain, at the surface and to some depth as well, a considerable number of actinomyces. The number of these organisms increases especially when the bog is drained and cultivated. It is believed that the actinomyces take an active part in the decomposition of some of the resistant complexes in the peat and lead to the liberation of the nitrogen in an available form.

Lowmoor peats have a vigorous flora of nitrifying and cellulose-decomposing bacteria. These are largely responsible for the abundant formation of nitrates in the upper layers of the peat and the almost complete lack of celluloses among the chemical peat constituents. When a lowmoor peat is moistened with ammoniacal solution, it forms a very excellent medium for the formation of nitrates, provided the compost is properly aerated and the reaction is not allowed to become too acid. Fungi are found to be abundant in lowmoor peats, but only at the very surface of the undrained bog.

Highmoor peats, however, because of their high acidity are free from nitrifying and aerobic cellulose-decomposing bacteria as well as actinomycetes. They contain a highly specific bacterial flora, partly aerobic, partly facultative anaerobic, and partly obligate anaerobic, which grows readily at pH 4.0, a phenomenon not observed commonly among soil bacteria. A large number of these acid-resisting bacteria have been isolated from the various depths of the Maine profiles and a detailed description of these will follow later.

Omeliansky (28) found in lake mud or sedimentary peat formations the presence of the following groups of bacteria: proteolytic, denitrifying, anaerobic nitrogen-fixing, pectin-fermenting, aerobic cellulose-decomposing, anaerobic cellulose-decomposing, and fat splitting. He came to the conclusion that sedimentary peat (Sapropel) represents a medium in which various bacterial processes take place energetically, leading to the decomposition of proteins, carbohydrates, and fats.

As to the animal population of peat bogs, we find that an abundant fauna of rhizopods has been recorded to be present in sphagnum peats, some of the organisms preferring this habitat (18, 17). Distinct differences were observed between different types of sphagnum (37). Fully developed highmoors possess a definite, frequently abundant, flora of *Amphitrema* species (*flavum* and *wrightianum*). These may be missing in non-fully developed peats, but *Hyalosphenia papilio* and *H. elegans* are common to both. Highmoor peats contain also considerable numbers of protozoa not commonly found on sphagnum itself.

A study of a number of samples of sphagnum peat revealed (17) the following groups of Rhizopoda: (a) Species of *Diffugia*, *Centropyxis*, *Arcella*, *Nebela*, *Euglypha*, *Assulina*, *Corythion*, and *Trinema*. This group is recognized as belonging to the "forest-moss type," although distinctly sphagnum types, such as *Nebela militaris*, are also found here. (b) Some species of group a and, in addition, *Hyalosphenia papilio* and *H. elegans*; a more constant and regular fauna, known as the "*Hyalosphenia* type." (c) In addition to the above, *Amphitrema* species are present; fauna of this type is richest in kinds and is known as the "*Amphitrema* type." (d) *Quadrula symmetrica* predominates, also *Cyphoderia* and *Nebela* species. In general, the types of rhizopod associations found in peat bogs correspond to definite botanico-geological peat types. The abundance of fossil rhizopods in peat was pointed out by Lagerheim (23) and others. Many of these species are found in a fossil condition in the various layers of peat in quite considerable numbers.

The great abundance of algae in certain peat formations, frequently even forming special types of peat, has been established by various investigators (15) and need not be discussed here. The presence in peat of a considerable fauna of nematodes, oligochaetes, myriapods, insects, and various other invertebrates has also been established (17).

EXPERIMENTAL

Occurrence of microorganisms in natural peat formations

The nature and chemical composition of the peats used in the following investigations have been described in detail previously (41). Two types of lowmoor peat profiles, one from New Jersey (41) and one from the Everglades, Florida (42), and two highmoor peat profiles from Maine (43) were used for this purpose.² In the case of the New Jersey and Maine peats, samples were taken by the authors into sterile glass containers and analyses immediately made when the samples arrived at the laboratory.

A synthetic medium (egg-albumin agar) was employed in determining the numbers of bacteria by the plate method; the abundance of anaerobic bacteria

TABLE 1

Occurrence of microorganisms at different depths of a lowmoor peat profile from Newton, N. J.
On the basis of fresh peat material

| DEPTH OF SAMPLE | pH | MOISTURE CONTENT | BACTERIA (AEROBIC AND FACULTATIVE AEROBIC AND ACTINOMYCES) | ACTINOMYCES | FUNGI | AEROBIC CELLULOSE DECOMPOSING BACTERIA* | NITRIFYING BACTERIA* | ANAEROBIC BACTERIA* |
|-----------------|-----|------------------|--|-------------|---------|---|----------------------|---------------------|
| cm. | | per cent | | per cent | | | | |
| Surface | 5.9 | 61.1 | 6,000,000 | 90 | 105,000 | ++ | ++++ | + |
| 30 | 6.0 | 72.5 | 350,000 | 40 | 250 | + | ++ | ++ |
| 45 | 6.2 | 82.3 | 450,000 | 25 | 175 | 0 | ++ | ++ |
| 60 | 6.3 | 87.5 | 40,000 | 20 | 150 | 0 | + | ++ |
| 75 | 6.3 | 87.1 | 35,000 | 25 | 33 | 0 | + | ++ |
| 90 | 6.4 | 80.8 | 20,000 | 15 | 0 | 0 | 0 | ++ |
| 120 | 6.7 | 83.6 | 100,000 | 2 | 0 | 0 | 0 | ++++ |
| 150 | 6.8 | 84.5 | 500,000 | 0 | 0 | 0 | 0 | +++++ |
| 165 | 8.0 | 64.8 | 200,000 | 0 | 0 | 0 | 0 | +++++ |
| Clay bottom | | | | | | | | |

* + designates a few; ++ a fair number; +++ abundance of organisms; ++++ numerous (about 25,000 or more colonies formed by 1 gm. of material).

was measured by the use of the same medium in a shake tube. For demonstrating the presence of cellulose-decomposing bacteria, tubes with liquid medium containing strips of cellulose as the only source of energy were found to be quite convenient (9).

Tables 1, 2, 3, and 4 give the numbers of aerobic and facultative anaerobic bacteria, per cent of actinomycetes, numbers of fungi, abundance of anaerobic

² The samples from the Everglade profile were taken under sterile conditions by Dr. R. V. Allison of the Belle Glade Station (Florida), to whom the authors are indebted for this courtesy. The authors wish to acknowledge here again their indebtedness to Dr. A. P. Dachnowski-Stokes, of Washington, D. C., for cooperation in the taking of the samples from the Maine profiles.

bacteria, cellulose-decomposing and nitrifying bacteria in 1-gm. portions of fresh peat taken at different depths of the different profiles.

The results of the microbiological analysis of the lowmoor peat profile from Newton, N. J., show an abundant development of bacteria throughout the whole profile, from the surface to the underlying clay. The greatest numbers of aerobic and facultative aerobic bacteria occur at the very surface and diminish rapidly with depth. Below a certain depth, namely, at about 90 cm., however, the numbers of bacteria begin to increase, largely because of the increase of anaerobic forms. This is well brought out in the results of the shake tube method, where the anaerobic bacteria are found to increase rapidly with depth. These tubes were prepared from a dilution of 1 to 50; numerous colonies of anaerobic bacteria were found in the tubes prepared from the lower parts of the profile. Among these bacteria the butyric acid organisms were quite abundant. It is to be recalled in this connection that only a small proportion of the viable anaerobic bacteria are capable of developing into colonies.

TABLE 2

Occurrence of microorganisms at different depths of a lowmoor peat profile from the Everglades, Fla.
On the basis of fresh peat material

| DEPTH OF SAMPLE | pH | MOISTURE | BACTERIA (AEROBIC AND FACULTATIVE AEROBIC AND ACTINOMYCES | ACTI- NOMYCES | FUNGI | CELLULOSE DECOM- POSING BACTERIA | NITRIFYING BACTERIA | ANAEROBIC BACTERIA |
|--------------------|-----|----------|---|------------------|--------|---|------------------------|-----------------------|
| cm. | | per cent | | per cent | | | | |
| Surface | 6.2 | 66.8 | 9,600,000 | 35 | 26,000 | ++ | +++ | + |
| 26-40 | 6.4 | 71.4 | 32,800,000 | 22 | 2,000 | ++ | ++ | ++ |
| 50-62 | 6.5 | 85.0 | 3,000,000 | 23 | 0 | ++ | ++ | ++ |
| 110-120 | 6.3 | 83.4 | 1,600,000 | 0 | 0 | + | + | +++ |

The actinomyces and fungi are very numerous at the surface of the peat and diminish rapidly with depth. The same is true of the nitrifying and aerobic cellulose-decomposing bacteria. It is interesting to note that the rapid drop in the abundance of nitrifying and cellulose-decomposing bacteria, fungi, and actinomyces was quite parallel with the drop in the total numbers of bacteria as determined by the plate method. On the other hand, the increase in the number of bacteria at a depth below 90 cm. was accompanied by an increase in the abundance of anaerobic bacteria, by a total lack of aerobic cellulose-decomposing and nitrifying bacteria, by the disappearance of fungi, and by a rapid drop in the numbers of actinomyces.

An attempt was also made to determine the abundance of *Bact. radiobacter* cells, by plating out the peat on glycerine-nitrate soil extract agar containing crystal violet, in concentration of 1 gm. of dye to 100,000 parts of medium. About 275,000 cells of *Bact. radiobacter* were found per gram of fresh surface peat. The numbers then diminished rapidly with depth and disappeared altogether at 90 cm.

These results indicate that from the surface to a depth of 90 cm. an aerobic flora prevailed in this particular peat formation, although it diminished rapidly below the surface few centimeters. At depths lower than 90 cm., the aerobic

TABLE 3

Occurrence of microorganisms at different depths of a highmoor peat profile from Cherryfield, Me.
On the basis of fresh material

| DEPTH OF SAMPLE | pH | MOISTURE CONTENT | AEROBIC AND FACULTATIVE ANAEROBIC BACTERIA PER GRAM | ACID-RESISTING ANAEROBIC BACTERIA |
|-----------------|------|------------------|---|-----------------------------------|
| <i>cm.</i> | | <i>per cent</i> | | |
| Surface layer | | | 250,000 | 0* |
| 7.5-20 | 4.05 | 92.7 | 100,000 | + |
| 20-30 | 3.95 | 92.6 | 220,000 | + |
| 30-45 | 3.85 | 92.6 | 1,600,000 | + |
| 45-60 | 3.86 | 92.9 | 3,500,000 | ++ |
| 60-75 | 3.73 | 93.6 | 1,500,000 | ++ |
| 120-150 | 3.90 | 93.6 | 2,100,000 | +++ |
| 175-210 | 4.47 | 93.4 | 750,000 | ++ |
| 450-480 | 4.71 | 92.4 | 800,000 | +++ |
| 540-570 | 5.18 | 92.2 | 2,000,000 | ++++ |

* The determinations of the numbers of anaerobic bacteria were carefully repeated using dilutions prepared so as to enable a more accurate counting of the organisms; 15,000 cells of bacteria capable of developing into colonies were found in each gram of moist peat in the 20-30 cm. layer, 550,000 in the 45-60 cm. layer, and 350,000 in the 120-150 cm. layer.

TABLE 4

Occurrence of microorganisms at different depths of a sphagnum peat profile from Orono, Me.
On the basis of fresh material

| DEPTH OF SAMPLE | pH | MOISTURE CONTENT | AEROBIC AND FACULTATIVE ANAEROBIC BACTERIA PER GRAM | ACID-RESISTING ANAEROBIC BACTERIA |
|-----------------|------|------------------|---|-----------------------------------|
| <i>cm.</i> | | <i>per cent</i> | | |
| 2.5-10 | 4.35 | 94.2 | 100,000 | ++* |
| 15-20 | 4.30 | 93.9 | 120,000 | + |
| 22.5-30 | 3.95 | 91.8 | 260,000 | + |
| 90-120 | 4.13 | 93.7 | 650,000 | ++ |
| 150-180 | 4.20 | 95.0 | 750,000 | +++ |
| 240-270 | 5.70 | 92.6 | 1,250,000 | ++ |
| 270-330 | 6.04 | 89.9 | 2,000,000 | +++ |

* Repeated determinations of the number of anaerobic bacteria, by the shake tube method, using only two samples taken at the 15-20 cm. and at the 90-120 cm. levels, gave 180,000 and 190,000 cells capable of developing into colonies per gram of moist peat.

flora disappeared completely and was replaced by an anaerobic flora. Attention should be called here to the fact that this bog underwent a certain amount of draining, as shown by the comparatively low moisture content of the peat within the upper 45 cm.

The results of the microbiological analysis of the lowmoor peat profile from Florida (table 2) are very similar to the results obtained in the analysis of the Newton profile, with certain minor differences. Here as well, one finds a very extensive bacterial flora from the surface of the bog to a depth of 40 cm. Below this layer the numbers, as shown by their development on the ordinary standard agar plate, diminish. The actinomyces, fungi, and aerobic cellulose-decomposing and nitrifying bacteria also diminish with depth, and their greatest abundance coincides with the greatest development of aerobic bacteria as determined by the plate method. The anaerobic bacteria, in this peat as well, increase with depth.

The occurrence and abundance of microorganisms in the sphagnum peat profiles present a distinctly different picture from that found in the two lowmoor peats. The sphagnum peats, being very acid in reaction, would naturally not be expected to contain many, if any at all, of certain types of bacteria; one would not expect to find, for example, organisms like the cellulose-decomposing bacteria, the nitrifying bacteria, and the nitrogen-fixing *Azotobacter*, which are known (40) to have a lower acid limit (higher pH value) than that found in these peats. A review of the previous literature on the occurrence of bacteria in natural highmoor peat formations actually points to the absence of *Azotobacter* and of nitrifying bacteria in these peats. The results of a microbiological study of the sphagnum peat profiles from Maine fully confirm those observations (tables 3 and 4). The nitrifying and aerobic cellulose-decomposing bacteria were found to be lacking altogether. The fungi were present at the very surface of the bog but not below the surface layer. Actinomyces were also lacking almost entirely. The bacteria developing on the plate were largely anaerobic or facultative anaerobic in nature. Contrary to the general expectations, the numbers were found to increase rapidly with depth, as shown both by the plate and the shake tube method. Most of the bacteria were acid resistant as shown by their growth in a medium (11) of pH 4.0, which was used for determining the numbers of these organisms by the shake tube method. The anaerobic bacteria developed in this medium very abundantly, producing gas within 20 to 48 hours. Hundreds of colonies were found in each tube of dilutions of 50 to 500 made of the peat taken even from the lowest depth of the profile. Among these anaerobic organisms, butyric acids and alcohol-forming bacteria were found to occupy a prominent place. However, cellulose-decomposing bacteria were lacking almost entirely or were found only in a few isolated instances. Most of the bacteria found in the lower layers were capable of using only sugars and proteins as sources of energy.

Quite similar results were obtained from two other sphagnum profiles investigated. This proves conclusively not only the existence of an abundant bacterial flora in the whole sphagnum peat profile, but also that this flora is quite specific, depending on the nature of the peat and upon the composition and depth of the particular horizon.

Decomposition of peat by microorganisms

The rôle of bacteria in the slow but gradual transformation of peat which has been laid down many years ago is still a matter of dispute. Some investigators claim (29, 39) that anaerobic bacteria are constantly at work decomposing the proteins and celluloses of the peat; others (19) deny entirely any possible action of bacteria and fungi, once the organic residues have been laid down under water. This may be either because of the lack of proper organisms, the unfavorable conditions under which the decomposition processes have to take place, or the resistance of the particular plant residues to attack by those organism which are capable of living under the particular conditions. Melin et al (26) found, for example, that when peat is allowed to undergo a process of fermentation, using sewage sludge as an inoculum, only the celluloses and hemicelluloses are decomposed but not the lignins or the so-called humic acids.

From the point of view of practical utilization of peat, the decomposition processes which result in the liberation of the constituent elements, especially of the nitrogen and carbon, in forms available for plant growth are of prime importance. The evolution of carbon dioxide is a good index for measuring the rapidity of decomposition of peat material, while the formation and accumulation of ammonia and nitrate can be used for measuring the rapidity of liberation of the nitrogen in an available form.

It has been shown elsewhere (44) that in the decomposition of grasses and cereal straw, as well as of sedges and reeds, none of the nitrogen in the plant is made available during the early stages of decomposition. As a matter of fact a considerable amount of additional combined inorganic nitrogen may be required for a rapid disintegration of the plant materials to take place. This is because the bacteria and fungi bringing about the decomposition of the celluloses and of the hemicelluloses consume large quantities of nitrogen for the synthesis of their cell substance, these synthetic processes being rendered possible through the presence of energy thereby made available. A large part of the plant nitrogen and the additional inorganic nitrogen are thus changed into organic nitrogenous compounds of microbial origin. As a result of this, the lowmoor peat is found to contain more nitrogen than the plants from which the peat has originated.

On the other hand, in the case of the sphagnum plants, the celluloses and hemicelluloses are not readily acted upon, under the anaerobic conditions prevailing in the bog, by microorganisms and hence offer only limited amounts of available energy for the building up of the microbial cell substance and the numerous microbial activities. Largely as a result of this, a part of the nitrogen liberated in the process of decomposition of the nitrogenous complexes of the dead sphagnum is thus left unassimilated by the microorganisms, and can be used by the growing plants. This accounts largely for the fact that the sphagnum peat may have less nitrogen than the living sphagnum plants.

The high cellulose and hemicellulose content of sphagnum peats serves as a further confirmation of these observations.

The same process of reasoning can be applied in an attempt to offer an explanation for the results of Kupreenko and Logvinova (24) who found that sphagnum peat contains a large quantity of its nitrogen (usually 11 to 14 per cent, and frequently as much as 19 to 28 per cent, of the total nitrogen) in the form of adsorbed ammonia, whereas lowmoor peat contains only traces of nitrogen in this form. When a comparison was made of the availability of nitrogen in the two forms of peat, using vegetation experiments, the sphagnum peat proved to be, during the first year growth of the plants, a much better

TABLE 5

Influence of depth of a lowmoor peat upon its decomposition at a constant moisture and air supply
100-gm. portions of moist peat incubated at 25–28°C. for 50 days

| DEPTH OF SAMPLE | MOISTURE CONTENT | TOTAL CO ₂ LIBERATED AS CARBON | AMMONIA FORMED | NITRATE FORMED | TOTAL NITROGEN LIBERATED |
|-----------------|---------------------|---|----------------|----------------|-----------------------------|
| cm. | per cent | mgm. | mgm. of N | mgm. of N | mgm. of N |
| Surface | 61.1 | 98.7 | 0.40 | 8.10 | 8.50 |
| 60 | 87.5 | 118.2 | 3.78 | 0.60 | 4.38 |
| 120 | 83.6 | 36.4 | 1.98 | 0.30 | 2.28 |
| 165 | 64.8 | 63.2 | 1.60 | 0 | 1.60 |

TABLE 6

Decomposition of sterile lowmoor peat (20 gm. dry matter), by different microorganisms, in 28 days

| INOCULUM | CO ₂ LIBERATED | AMMONIA FORMED | NITRATE FORMED | TOTAL NITROGEN LIBERATED | RATIO OF C/N LIBERATED |
|--------------------------|------------------------------|-------------------|-------------------|--------------------------------|------------------------------|
| | mgm. of C | mgm. of N | mgm. of N | mgm. | |
| Soil suspension..... | 68.7 | 5.8 | 3.8 | 9.6 | 7.16 |
| <i>Trichoderma</i> | 88.4 | 13.3 | 0.8 | 14.1 | 6.13 |
| <i>Actinomyces</i> | 87.7 | 11.6 | 1.8 | 13.4 | 6.54 |

source of nitrogen than the lowmoor peat. This phenomenon was naturally explained by the high content of adsorbed ammonia in the sphagnum peat, but no explanation was suggested for the origin of this ammonia. A study of the process involved in the decomposition of the two types of peat material would lead us to expect this very phenomenon to take place.

A comparison of the nitrate formation from lowmoor peats and from highmoor peat properly limed also brought out the fact (24) that, although the former contains much more nitrogen than the latter, this nitrogen is much more inert. Only 2.6 per cent of the total nitrogen was changed to nitrate in the case of a lowmoor peat composted for a period of five months, and 5 per cent in nine months; in the case of the highmoor peat, 9 per cent of the total nitrogen was changed to nitrate within two months, in the presence of enough CaO to bring the reaction to pH 6.5.

To measure the decomposition processes taking place in lowmoor peats, 100-gm. portions of fresh, moist Newton peat taken at four different depths were introduced into a series of flasks, placed in the incubator at 25–28°C., and connected with the aeration apparatus. Table 5 shows the rapidity of decom-

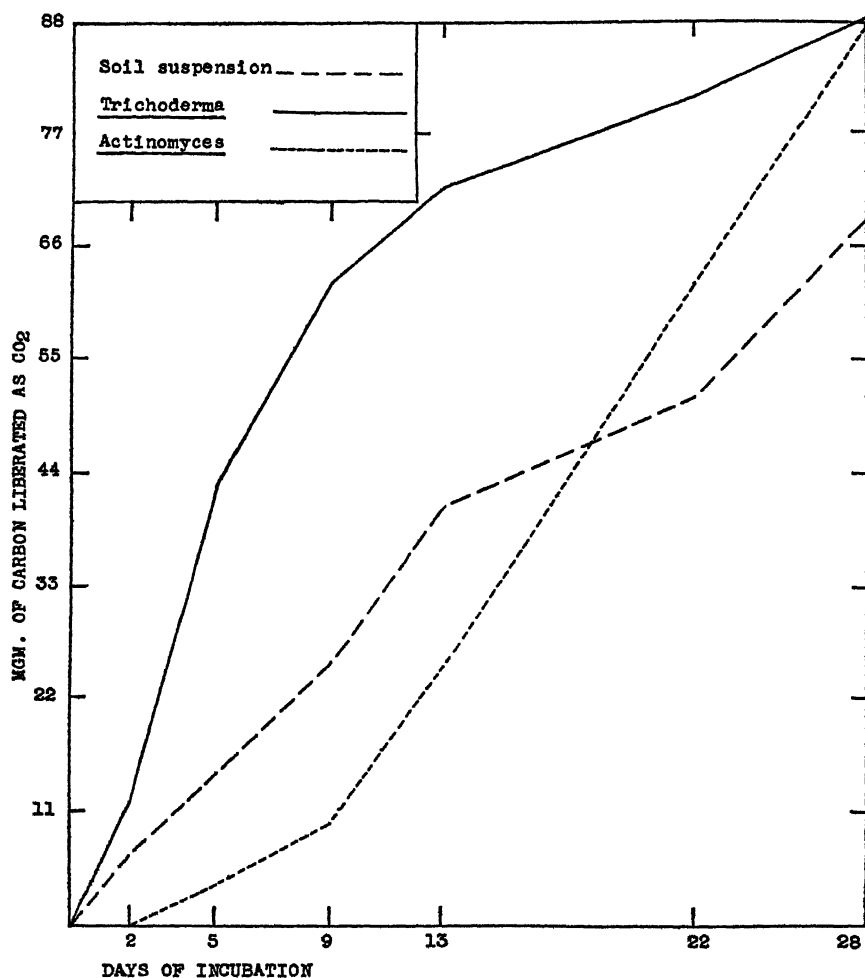


FIG. 1. COURSE OF DECOMPOSITION OF STERILE LOWMOOR PEAT BY A SOIL SUSPENSION AND BY PURE CULTURES OF MICROORGANISMS, AS MEASURED BY THE LIBERATION OF CO₂

position of 100-gm. portions of moist peat taken from different depths, as shown by the amount of carbon liberated as CO₂ and of nitrogen liberated as ammonia and nitrate, in a period of 50 days. The results show that in general the surface layers decompose with the greatest rapidity.

To determine the effect of the microbiological population upon the decomposition of peat, 70-gm. portions of Newton peat containing 20 gm. of dry matter were placed in flasks and sterilized at 15 pounds pressure for two hours. The flasks were then inoculated with a soil suspension or with pure cultures of the fungus *Trichoderma* or a typical soil *Actinomyces*. The amounts of CO₂ produced and of nitrogen liberated in 30 days are recorded in table 6, and the course of decomposition of the sterile peat by the two pure cultures and the soil suspension is given in figure 1. These results show that pure cultures of microorganisms, such as *Trichoderma* or *Actinomyces*, are capable of decomposing peat just as actively as and even more so than a mixed soil suspension. This is true in the case both of CO₂ production and of liberation of available nitrogen. It is interesting to note that nitrates were formed in

TABLE 7

Influence of inoculation, addition of inorganic nutrients and organic materials upon the decomposition of fresh lowmoor peat material

| TREATMENT | CO ₂ GIVEN OFF, MILLIGRAMS OF CARBON | | | | | | NH ₃ -N | NO ₃ -N | TOTAL N LIBER- ATED |
|--|---|-------|-------|-------|-------|-------|--------------------|--------------------|---------------------------|
| | Days of incubation | | | | | | | | |
| | 6 | 10 | 15 | 24 | 33 | 39 | | | |
| | | | | | | | mgm. | mgm. | mgm. |
| Uninoculated..... | 20.4 | 33.9 | 49.3 | 68.4 | 89.5 | 100.2 | 0.45 | 7.45 | 7.90 |
| Suspension of fertile soil..... | 22.7 | 36.5 | 54.4 | 73.7 | 95.1 | 106.8 | 1.17 | 6.72 | 7.91 |
| Suspension of fresh cow ma- nure..... | 23.6 | 38.3 | 56.8 | 76.9 | 100.8 | 116.7 | 0.97 | 8.91 | 9.88 |
| Mixture of <i>Actinomyces</i> | 20.8 | 34.3 | 52.6 | 72.7 | 94.3 | 106.6 | 1.70 | 8.71 | 10.41 |
| Green <i>Trichoderma</i> | 21.8 | 36.3 | 53.4 | 73.5 | 96.3 | 108.3 | 2.67 | 7.77 | 10.44 |
| 400 mgm. (NH ₄) ₂ SO ₄ + 100 mgm. K ₂ HPO ₄ | 26.7 | 43.9 | 64.0 | 83.6 | 103.6 | 115.7 | 73.81 | 6.58 | |
| Cellulose, 2 gm..... | 37.4 | 100.4 | 161.9 | 218.1 | 270.4 | 308.8 | 0.40 | 0.85 | 1.25 |
| Rye straw, 2 gm..... | 63.1 | 129.4 | 192.5 | 256.7 | 321.5 | 364.6 | 0.34 | 0.48 | 0.82 |

appreciable amounts only when the soil suspension was used as an inoculum. In the case of the *Trichoderma* and *Actinomyces*, all the nitrogen liberated in the decomposition of peat was in the form of ammonia. The small amounts of nitrate found in these cultures were largely present originally in the peat.

To throw further light upon the influence of inoculation as well as the addition of available salts and undecomposed organic matter upon the decomposition processes in natural fresh peat, another experiment was carried out. In a series of flasks were placed 100-gm. portions of fresh Newton peat containing 28 gm. of dry matter. These were variously inoculated. Some flasks received the addition of ammonium sulfate (80 mgm. of nitrogen) and dipotassium phosphate (100 mgm.). Others received 2-gm. portions of cellulose or rye straw. The flasks were placed in the incubator, connected with the aeration apparatus, and incubated for 39 days. At the end of the in-

cubation period, the amounts of ammonia and nitrate as well as the numbers of bacteria (by the plate method) were determined (table 7).

The uninoculated peat was found to contain 7,500,000 cells of microorganisms, developing on the plate, per gram, of which 80 per cent were actinomycetes. There were also found 80,000 fungi per gram of peat. Inoculation with manure brought about an increase in the number of bacteria (probably due to their actual introduction with the manure) to 11,000,000 per gram, of which 65 per cent were actinomycetes. The addition of a suspension of fertile soil did not modify to any considerable extent the bacterial and actinomycetes population in the peat, but brought about a certain modification in the fungous population. The addition of nutrients increased somewhat the numbers of bacteria and reduced the numbers of fungi.

The fact that the addition of available nitrogen, phosphorus, and potassium did not bring about any appreciable increase in the evolution of CO_2 points definitely to the fact that nitrogen is not a limiting factor in the activities of microorganisms in peat but that the available carbon compounds are. When available energy, in the form of cellulose or rye straw, is added to the peat, rapid decomposition sets in, as indicated by the rapid increase in the evolution of CO_2 . The addition of cellulose in the form of filter paper brought about a rapid increase in the numbers of fungi (species of *Cephalosporium*, *Fusarium*, *Trichoderma*, *Humicola*, *Penicillium*, but no Mucorales). When rye straw was added, the Mucorales, in addition to other fungi, developed quite abundantly. Various cellulose-decomposing bacteria, including *Spirochaeta cytophaga*, a very common form, developed in great abundance as a result of the addition both of cellulose and of straw. These results lead us to conclude that lowmoor peat is abundantly supplied with all microorganisms required for its rapid decomposition and for the liberation of its nitrogen in the form of nitrate. The organic complexes in the peat are very resistant to decomposition, as indicated by the fact that when only 2 gm. of cellulosic material is added to 100 gm. of peat containing 28 gm. of dry material there was three times as much carbon dioxide produced. This points to the comparative rapidity of decomposition of filter paper and straw, on the one hand, and to the slow decomposition of the organic complexes in peat, on the other. When cellulose and straw were added, there was practically no nitrogen left in the peat mixture, either as ammonia or as nitrate. This is because of the reassimilation of the available nitrogen by the microorganisms which use the cellulose and the straw as sources of energy.

To compare the results of the rapidity of peat decomposition with the decomposition of some of the plant materials which contribute to the formation of the peat, the stems (and leaves) and the roots or rhizomes of *Carex* and of *Cladium* (saw-grass) have been submitted to decomposition. A moisture content of 200 per cent, a nutrient solution containing ammonium phosphate and potassium chloride, and a soil suspension for inoculation were used. Twenty-gram portions of the dry material of *Carex* were placed in flasks and

40 cc. of water added. In the case of the *Cladium*, only 2-gm. portions were added to 100 gm. of quartz sand containing 20 cc. of water and the nutrient solution. The results of the evolution of CO_2 from the decomposition of these materials are given in figure 2.

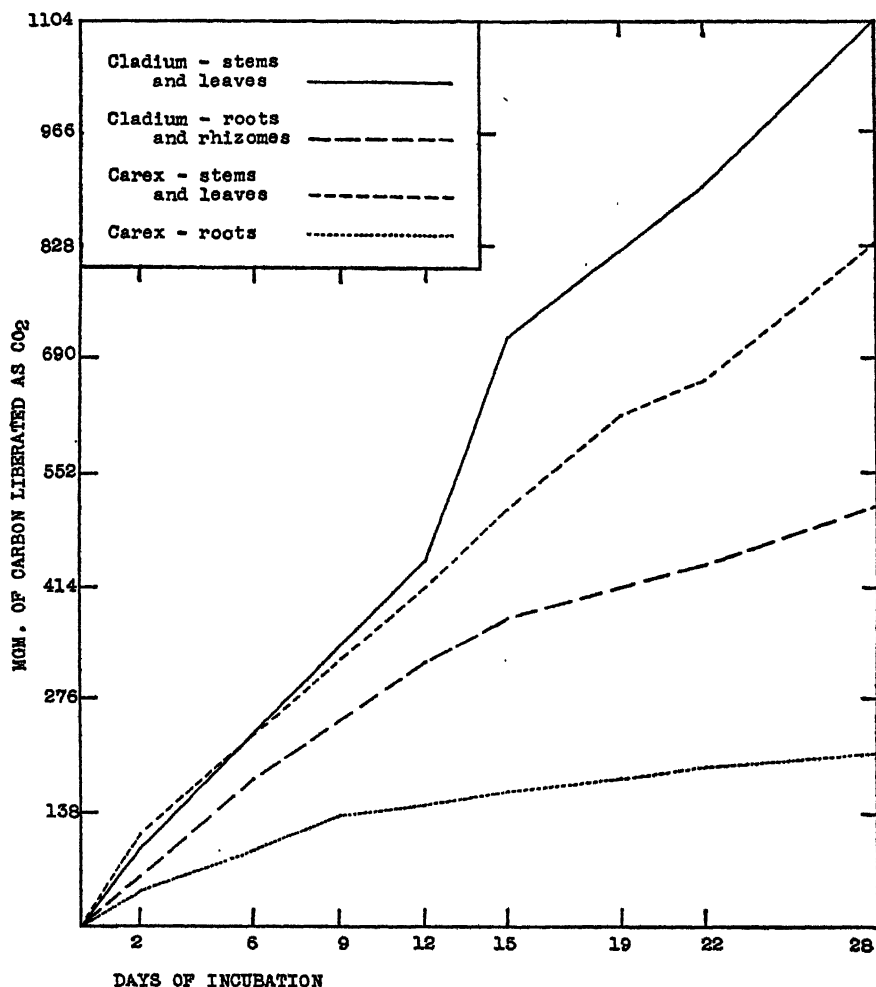


FIG. 2. DECOMPOSITION OF STEMS (AND LEAVES) AND ROOTS (AND RHIZOMES) OF *CAREX* (20 GM. OF DRY MATTER) AND *CLADIUM* (2 GM. OF DRY MATTER IN SAND CULTURES) PLANTS

In both cases, the stems and leaves decomposed much more rapidly than the roots and rhizomes. An analysis of these materials (44) shows that the stems and leaves are considerably richer in celluloses and pentosans than the roots, whereas the latter are much richer in lignins, which accounts for the difference

in the rapidity of decomposition. In the case of the *Cladium* stems, 5.2 mgm. of ammonia nitrogen was consumed from the inorganic solution, whereas in the case of the *Cladium* roots only 0.4 mgm. of ammonia nitrogen was used, because of less decomposition of the roots and greater protein content.

To determine the rapidity of decomposition of highmoor or sphagnum peat, two horizons were used: a light brown layer of younger sphagnum and a dark brown layer of older sphagnum peat of an Oldenburg profile from Germany, described elsewhere (41). Twenty-gram portions of dry peat and 40-cc. portions of tap water were introduced into a series of flasks; various salts were then added and the peat was incubated for 24 days. The amount of CO_2 produced was used as an index of the rapidity of decomposition of the peat.

Both the younger and the older sphagnum peats decomposed (table 8) less rapidly than the lowmoor peats. The older sphagnum decomposed somewhat

TABLE 8
Decomposition of 20 gm. portions of younger and older sphagnum peat from an Oldenburg peat profile

| TREATMENT OF PEAT | CO ₂ LIBERATED, MGM. OF CARBON | | | | | |
|--|---|------|------|---------------|------|-------|
| | Young sphagnum* | | | Old sphagnum† | | |
| | Days of incubation | | | | | |
| | 5 | 10 | 24 | 5 | 10 | 24 |
| Untreated..... | 8.6 | 16.4 | 29.4 | 8.7 | 18.4 | 33.6 |
| 500 mgm. (NH ₄) ₂ HPO ₄ + 100 mgm. K ₂ HPO ₄ | 14.1 | 22.8 | 38.2 | 12.2 | 20.8 | 36.7 |
| 1 gm. CaCO ₃ | 41.3 | 56.6 | 88.3 | 53.5 | 72.3 | 108.8 |
| 50 mgm. CuSO ₄ | 8.6 | 17.9 | 36.1 | 10.8 | 19.4 | 35.2 |

* pH of peat 4.1, nitrogen content, 0.83 per cent.

† pH of peat 4.4, nitrogen content 0.99 per cent.

more rapidly than the younger; it was less influenced by the addition of available nitrogen salt, but more so by the addition of CaCO_3 . The large amount of CO_2 liberated in the presence of CaCO_3 is due chiefly to the chemical interaction between the organic acid reacting complexes and the carbonate. The addition of CuSO_4 seemed to have no effect upon the rapidity of decomposition of both layers of peat.

To establish the rapidity of decomposition, a sphagnum peat from Maine (Cherryfield) described elsewhere (43), recently taken from the bog, with and without additional inorganic salts and carbonate, was used. Sufficient air-dry peat was used to give 25 gm. of dry material; enough water was added to bring it to 300 per cent moisture. The peat was placed in aeration flasks, inoculated with fresh soil suspension and incubated at 25–27°C. for 19 days. The amount of CO_2 liberated was determined at frequent intervals. At the end of the incubation period, the amounts of nitrogen liberated in an inorganic

form (ammonia + nitrate) were determined. Only traces of nitrates were produced in all flasks. The results are given in table 9.

Here as well, the addition of ammonium sulfate and dipotassium phosphate had no stimulating effect upon the processes of decomposition. This is primarily because in the sphagnum peat, the nitrogenous complexes decompose rapidly, whereas the carbohydrates, which are quite abundant in this type of peat (44), are quite resistant to decomposition. The ratio between the carbon liberated as CO_2 and the nitrogen liberated as ammonia and nitrate is narrow in the case of the lowmoor and sedimentary peats, ranging from 12:1 to 13:1 in the case of the Newton peat, to 3:1 for the saw-grass peat in Florida, and only about 1.3:1 for the sedimentary peat. In the case of the highmoor or sphagnum peat, however, this ratio is about 20:1. The only exception in respect to this ratio is found in the lowmoor peats taken from lower depths. The results presented in table 5 show that the deeper the layer of peat in the bog, the wider is the ratio between the CO_2 and the nitrogen liberated.

TABLE 9
Decomposition of sphagnum peat under different treatments

| TREATMENT | CO_2 LIBERATED | $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ |
|--|----------------------------|--|
| | mgm. C | mgm. N |
| Untreated | 94.4 | 4.65 |
| 100 mgm. $(\text{NH}_4)_2\text{SO}_4$ + 100 mgm. K_2HPO_4 | 86.6 | 18.95 |
| 0.5 gm. CaCO_3 | 125.1 | 4.55 |
| 1.0 gm. CaCO_3 | 146.3 | 4.25 |
| 1.0 gm. CaCO_3 + 100 mgm. K_2HPO_4 | 135.7 | 4.05 |
| 1.0 gm. CaCO_3 + 100 mgm. $(\text{NH}_4)_2\text{SO}_4$ + 100 mgm. K_2SO_4 | 134.1 | 18.30 |

To determine the influence of moisture upon the decomposition of peat, a part of the lowmoor peat used in the previous investigations was air-dried, then adjusted, by the addition of water, to various moisture contents. The drying of the peat made it in some manner less readily available to the action of microorganisms and less capable of absorbing moisture. Whereas natural lowmoor peat may contain as much as 92 per cent moisture and 8 per cent dry matter or over 1000 per cent moisture, the dried material could not absorb more than 400 per cent moisture and the excess water remained in a free state. It is possible that the drying results in a change in the physical condition of the hemicelluloses into horny-like substances; these may be irreversible in nature and may exert a protective effect upon the decomposition of the various complexes in the peat. Possibly the removal of this hemicellulose from the peat by the HCl treatment, as shown in the following experiments was one of the factors favoring its more rapid decomposition.

The influence of moisture content and addition of inorganic nitrogen upon the decomposition of peat is shown in table 10. Ammonia and nitrate were determined at the end of the incubation period, namely, after 20 days. The

addition of inorganic nitrogen exerted again only a minor influence upon the rapidity of decomposition, as shown by the evolution of CO_2 . The undried peat decomposed most rapidly. The air-drying of the peat had an injurious effect upon the rapidity of its decomposition and also upon the nitrifying bacteria. Whereas in the fresh peat the nitrogen liberated is rapidly changed to nitrate, in the dried peats most of the nitrogen liberated remained as ammonia. The lowest amounts of CO_2 were given off with the highest moisture content. In the presence of an excess of water, conditions became more and more anaerobic, favoring the activities of anaerobic bacteria. The lack of depression in the liberation of available nitrogen shows that even under partial

TABLE 10

Influence of moisture and additional nitrogen upon microbiological activities in lowmoor peat
On the basis of 20 gm. of dry peat

| MOISTURE CONTENT | NITROGEN ADDED* | CO_2 GIVEN OFF, MILLIGRAMS OF CARBON | | | | $\text{NH}_3 - \text{N}$ | $\text{NO}_3 - \text{N}$ | TOTAL NITROGEN |
|---------------------|--------------------|---|--------|---------|---------|--------------------------|--------------------------|-------------------|
| | | 2 days | 8 days | 12 days | 20 days | | | |
| <i>per cent</i> | | | | | | <i>mgm.</i> | <i>mgm.</i> | <i>mgm.</i> |
| Natural peat† | — | 14.8 | 34.5 | 46.8 | 64.3 | 0.3 | 11.3 | 11.6 |
| Natural peat‡ | + | 16.8 | 37.9 | 50.9 | 68.7 | 0.3 | 22.2 | 22.5 |
| 200‡ | — | 8.3 | 23.1 | 35.0 | 54.4 | 7.9 | 1.5 | 9.4 |
| 200 | + | 9.0 | 24.3 | 36.1 | 54.5 | 17.5 | 1.7 | 19.2 |
| 300 | — | 6.6 | 20.6 | 31.0 | 48.9 | 8.2 | 0.8 | 9.0 |
| 300 | + | 8.2 | 23.8 | 34.7 | 51.6 | 18.4 | 1.0 | 19.4 |
| 450 | — | 3.4 | 13.9 | 20.2 | 32.2 | 9.1 | 0.9 | 10.0 |
| 450 | + | 3.4 | 14.9 | 23.3 | 36.4 | 20.6 | 0.9 | 21.5 |

* 10 mgm. of nitrogen added in the form of ammonium sulfate.

† Fresh peat containing 67 per cent moisture.

‡ Peat previously air-dried, then adjusted to 200, 300, and 450 per cent moisture.

anaerobic conditions the nitrogenous complexes are readily decomposed. The higher the moisture content of the peat the narrower was the C:N ratio or the ratio between the carbon liberated as CO_2 and the nitrogen as ammonia.

Influence of peat treatment upon its decomposition by microorganisms

Wollny (47) found that on removing the waxes from peat with ether, the rapidity of its decomposition doubled. To throw further light upon this subject, a quantity of the lowmoor peat profile from Newton was divided into three portions. One portion was left untreated. The second portion was treated with toluene for 48 hours and the toluene allowed to evaporate. The third portion was heated with a solution of 2 per cent HCl , for one hour at 15 pounds pressure in the autoclave; the peat was then filtered and washed with tap water until practically free from acid; the remaining acidity was neutralized by the addition of a small amount of CaO .

Enough peat of each of the three preparations was introduced into a series of flasks to give 20 gm. of dry material; the moisture was then adjusted to 200 per cent. All flasks were inoculated with a suspension of good garden soil and incubated for 20 days at 25°C.

The results (table 11) show that treatment of peat with toluene greatly favored its decomposition, as shown by the evolution of carbon dioxide, the liberation of nitrogen in an available form, and the development of bacteria. The numbers of fungi diminished. The nitrifying organisms were injured somewhat, as a result of which most of the nitrogen was left in the form of ammonia. Treatment of the peat with dilute acid at a high temperature resulted in the removal of a large part of the protein and of the hemicellulose. Nevertheless the remaining material decomposed much more rapidly than the untreated peat, showing that either some protective substance or some injurious substance was removed by the treatment. It is interesting to note the number

TABLE 11

Influence of treatment of lowmoor peat upon the growth and activities of microorganisms
On basis of 20 gm. of dry peat (20 days)

| TREATMENT OF PEAT | UNTREATED | TOLUENE TREATED | HEATED WITH 2 PER CENT HCl |
|---|-----------|--------------------|----------------------------------|
| Mgm. of carbon liberated as CO ₂ | 64.3 | 95.3 | 69.0 |
| Mgm. of nitrogen liberated as ammonia..... | 0.3 | 21.1 | 17.1 |
| Mgm. of nitrogen accumulated as nitrate..... | 11.3 | 5.9 | 1.1 |
| Total nitrogen liberated, mgm..... | 11.6 | 27.0 | 18.2 |
| Numbers of bacteria in 1 gm. of moist peat..... | 8,300,000 | 46,000,000 | 7,000,000 |
| Numbers of fungi in 1 gm. of moist peat..... | 160,000 | 80,000 | 10,000,000 |

of fungi growing on the HCl-treated peat in comparison with the untreated and the toluene treated. The nitrifying bacteria were completely depressed and have not recovered as a result of inoculation, hence the nitrogen liberated was found entirely in the form of ammonia. Further experiments dealing with the influence of treatment of peat with ether, toluene, and dilute acid confirmed these observations.

Different treatments seem to influence differently the decomposition of the various organic complexes in the peat, as shown in table 12. The greatest effect upon the decomposition of the peat constituents was exerted by the ether treatment, as shown by the evolution of carbon dioxide. However, the lowest amount of nitrogen was liberated as a result of this treatment. This is no doubt because the non-nitrogenous complexes have been made, as a result of treatment with ether, more readily decomposable. Possibly some of the nitrogen might have been, as a result of that, reassimilated by the microorganisms, because of the very extensive development of both fungi and bacteria. Here as well, treatment with toluene and hot hydrochloric acid

resulted in an increase in the decomposition of the peat, as shown by the liberation of CO_2 and ammonia.

The fact that treatment with ether favors the decomposition of the non-nitrogenous complexes in peat is shown in table 13. Although the decomposition of highmoor peat was stimulated considerably on treatment with ether, as shown by the evolution of CO_2 , there was a reduction of the amount of available nitrogen liberated as ammonia, pointing to a reassimilation of a part

TABLE 12

Influence of treatment of lowmoor peat upon the growth and activities of microorganisms
On basis of 20 gm. of dry peat (22 days)

| TREATMENT OF PEAT | UNTREATED | ETHER TREATED | TOLUENE TREATED | HEATED WITH 2 PER CENT HCl |
|--|------------|---------------|-----------------|----------------------------|
| Mgm. of carbon liberated as CO_2 .. | 36.1 | 204.7 | 62.1 | 146.0 |
| Mgm. of nitrogen liberated as ammonia..... | 0.3 | 7.9 | 9.0 | 14.9 |
| Mgm. of nitrogen liberated as nitrate..... | 11.8 | 0.9 | 6.4 | 1.1 |
| Total nitrogen liberated..... | 12.1 | 8.8 | 15.4 | 16.0 |
| Numbers of bacteria and actinomycetes in 1 gm. of moist peat.. | 21,000,000 | 97,000,000 | 25,000,000 | 105,000,000 |
| Per cent of actinomycetes..... | 50 | 10 | 5 | 5 |
| Numbers of fungi in 1 gm. of moist peat..... | 235,000 | 7,075,000 | 51,000 | 359,000 |

TABLE 13

Influence of treatment of highmoor peat (Maine) upon its decomposition by microorganisms
On basis of 20 gm. of dry peat (21 days)

| TREATMENT OF PEAT | UNTREATED | 1 GM. CaCO_3 ADDED | 50 MGM. $(\text{NH}_4)_2\text{HPO}_4$ ADDED | TREATED WITH ETHER |
|--|-----------|-----------------------------|---|--------------------|
| Mgm. of carbon, liberated as CO_2 | 61.40 | 98.4 | 61.30 | 97.9 |
| Mgm. of nitrogen, as ammonia..... | 5.95 | 4.9 | 13.25 | 3.0 |

of the nitrogen by the microorganisms decomposing the carbonaceous complexes. The addition of available nitrogen had no effect upon the rapidity of decomposition of sphagnum peat.

The marked increase in the decomposition of peat following treatment with ether or with toluene, as measured by the evolution of CO_2 in the process of aerobic decomposition, points to some interesting generalizations. The removal of the fats and waxes, or ether-soluble substances, from lowmoor peat, brought about an evolution of CO_2 which was about five times as great as that of the untreated peat. The treatment of peat with toluene, even without the removal of the toluene extract but merely allowing the solvent to evaporate, more than doubled the rate of peat decomposition.

We can hardly explain this by the destruction of certain groups of microorganisms, such as protozoa, or by a stimulating effect of the disinfectant, but rather by a change produced in the chemical condition of the organic complexes of the peat. It was shown elsewhere that the removal of the ether-soluble material from fresh plant material, such as straw, favors its more rapid decomposition. We may expect similar results, probably even more marked, from the treatment of peat or of soil organic matter.

SUMMARY

1. Results of investigations on the occurrence and activities of microorganisms in different peat bogs are reported.

2. These results prove conclusively that microorganisms play a most important rôle in the formation of peat from the plant remains.

3. In lowmoor peat bogs the numbers of aerobic bacteria diminish rapidly with depth whereas the numbers of anaerobic bacteria increase rapidly with depth.

4. Fungi, aerobic cellulose-decomposing bacteria, and nitrifying bacteria are found in lowmoor peat bogs at or just below the surface of the bog, then diminish rapidly and disappear completely at a depth of 75 to 90 cm. Actinomyces are abundant at the surface of the lowmoor peat but they also diminish with depth but not so rapidly as the fungi; they disappear completely at a depth of 120-150 cm.

5. Acid sphagnum peat bogs contain an abundant flora of acid-resistant bacteria capable of growing in media of pH 4.0. In undrained sphagnum peat bogs, the numbers of bacteria, largely anaerobic forms, increase with depth, so that at a depth of 570 cm. there were found more bacteria growing on synthetic agar media than in the surface layers of the bog.

6. The rate of decomposition of peat, as shown by the evolution of CO_2 , is much slower than that of fresh plant residues.

7. With an increase in depth of peat there is a widening of the ratio (C/N) between the carbon liberated as CO_2 and the nitrogen liberated as ammonia and nitrate. The deeper the lowmoor peat the less active is its nitrifying capacity.

8. Certain pure cultures of fungi and actinomyces can decompose sterilized lowmoor peat as fast as the total soil population.

9. The addition of inorganic nitrogen salt and phosphates had practically no effect upon the rapidity of decomposition of lowmoor and highmoor peats, because available energy and not nitrogen is the limiting factor in the decomposition of peat material.

10. The ratio between the CO_2 and nitrogen liberated (C/N) in the course of peat decomposition is wider in the case of the highmoor sphagnum peats than in lowmoor peats.

11. Treatment of peat with ether, toluene, and dilute hydrochloric acid

followed by the removal of the reagent leads to a very marked increase in the rapidity of the peat decomposition.

12. The action of ether and toluene is not due so much to any change in balance of the microbial population of peat as to the removal of waxy substances rendering the peat more readily available for the action of microorganisms.

13. Different treatments differ markedly in the nature of their action upon peat, because different organic complexes in the peat are affected by each treatment.

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THE MICROFLORA OF LEACHED ALKALI SOILS: I. SYNTHETIC ALKALI SOIL

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A vast amount of work has been done on the effect of "alkali salts" upon the physical, chemical, and biological properties of soils. The biological work has dealt primarily with the chemical processes occurring in the soil, and little consideration has been given to the specific microorganisms which occur and function in such soils. It is generally known that actual qualitative and quantitative differences exist in the vegetation of "alkali" and "non-alkali soils," and it is not unreasonable to expect a similar variation in the microflora. Moreover, the unproductivity of recently leached alkali soil is probably due in a measure to the absence of the necessary microorganisms. For these reasons a rather comprehensive study has been made of the morphological and physiological properties of the microorganisms occurring in recently leached "alkali soils."

Three different soils were studied: (a) A synthetic "alkali soil," (b) a natural-occurring "alkali soil" rich in chlorides, and (c) a natural-occurring "alkali soil" rich in sulfates. This paper deals with the synthetic soil; the others will be considered in subsequent papers.

PREPARATION OF SOIL

The synthetic alkali soil was prepared by adding 0.66 per cent each of sodium chloride, sodium sulfate, and sodium carbonate to a naturally productive sandy loam. The moisture was made up to 20 per cent and maintained at this level for two months for the soil reactions to reach equilibrium. It was then leached for a period of 199 days, 240 liters of tap water passing through it (7). Analyses of the leachings showed that 91.3 per cent of the sodium chloride, 97.7 per cent of the sodium sulfate, and 43 per cent of the sodium carbonate were recovered. The leaching had increased the bacteria 237 per cent; the ammonifying powers, 336 per cent; the nitrifying powers, 3350 per cent; and the nitrogen-fixing powers, 357 per cent.

The pots were sampled and then seeded to crimson clover, and the plants and roots harvested at time of full bloom, after which the soil was again sampled

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and analyzed. It was found that the ammonifying powers of the leached soil had increased 4.7 per cent and the nitrifying powers, 40 per cent; the nitrogen-fixing powers had decreased 66 per cent, whereas the total number of colonies developing on agar had increased 31 per cent. The pots were then seeded to barley, which was harvested at the time of maturity, after which they were resampled and analyzed. The total number of colonies had decreased 28 per cent, the ammonifying powers had increased 9 per cent, the nitrifying powers had increased 34 per cent, and the nitrogen-fixing powers had decreased 11 per cent. A third crop of barley was grown on the soil, after which the samples used in this work were taken (6, 7).

Method of isolation. The total number of colonies developing on nutrient agar (8), synthetic agar (8), and Ashby agar (4) was determined as follows: The samples were air-dried and ground to pass through an 80-mesh sieve. Five grams of soil were added to 100 cc. of sterile water and shaken for five minutes. Further dilutions were made and platings made from dilutions of 1 to 20,000 and 1 to 200,000. The average from six determinations was:

| | |
|---------------------|-----------|
| Nutrient agar..... | 4,505,000 |
| Synthetic agar..... | 4,567,000 |
| Ashby agar | 2,808,000 |

There is a very close agreement between the number of colonies developing upon nutrient and synthetic agar, but the Ashby agar yielded only 61.5 per cent as many colonies as did the synthetic agar. For the pure-culture study, colonies were fished out of the Ashby agar plates and inoculated on Ashby agar slants, the Ashby media being selected for the following reasons: (a) Probably less work has been done on the colonies developing on this media than on the other two; (b) this soil was outstanding in its nitrogen-fixing powers; (c) many different organisms were evidently developing on this media, and the likelihood of getting the nitrogen fixers was greater than if the other media were used. The different colony types developing on Ashby agar were obtained in pure cultures by repeated dilutions, plating, and microscopic examination. The stock cultures were kept on soil extract Ashby agar. The soil extract was prepared by extracting 2 gm. of soil with 100 cc. of distilled water.

Ammonification. The ammonifying powers of the organisms were determined by inoculating each organism into 100 cc. of sterile 1 per cent peptone solution made from tap water and bacto-peptone. This was incubated for four days at 28°C. and then analyzed for ammonia by the Kjeldahl method, magnesium oxide being used as the base.

Urea decomposition was determined by inoculating 100-cc. portions of 1 per cent urea solution with the various organisms. These were incubated for four days at 28° and the ammonia determined.

Cellulose decomposition. All organisms were plated on cellulose agar (2). The degree of cellulose decomposition was determined by the area of the clear zone around the colonies. No. 7 failed to grow, and none of the organisms were able to decompose cellulose.

Nitrogen fixation. One-hundred-gram portions of finely ground garden soil were weighed into Erlenmeyer flasks and the moisture made up to 20 per cent with distilled water. These samples were autoclaved for two and one-half hours at 120°C., after which 10 cc. of distilled water, containing 1.5 gm. of mannitol, were added to each flask. The flasks were then inoculated in duplicate with each organism. Sterile checks were run. The samples were incubated for five weeks at 28°C., the moisture content being maintained at 26 per cent. At the end of three weeks of incubation an additional gram of mannitol was added, as a test had shown the initial amount to have been all used. At the end of five weeks the samples were dried at 120°C., ground, and the total nitrogen determined by the official Gunning method. Each culture was studied in detail, both morphologically and physiologically, according to the pure culture methods, but only a brief description of each organism is given. The ammonifying, urea-decomposing, and nitrogen-fixing powers are stated in milligrams as produced under the specifically described method. The organisms are probably not listed in Bergey's Manual (2) and are probably new species or varieties. They represent the microflora of this soil which will grow on nitrogen-free media and give a fair idea of the kind of organisms which develop on the Ashby media.

1A. Non-motile, gram-negative cocci 0.5 to 0.7 μ in diameter, occurring singly and in clusters. Grows well on all the ordinary laboratory media. Slowly liquefies gelatin. Abundant light tan growth on potato. Peptonizes milk with alkaline reaction. Produces indole. Does not reduce nitrates. Hydrolyzes starch but does not ferment other carbohydrates. Facultative aerobic. Produces 6.29 mgm. of ammonia and fixes 1.4 mgm. of nitrogen in soil.

1B. Non-motile, gram-negative, sporulating, rods 0.8 to 1.2 by 1.2 to 1.8 μ , occurring singly in pairs and clusters. Grows well on all ordinary laboratory media. Abundant tan growth on potato. Rapid gelatin liquefier. Slow peptonization of milk with alkaline reaction. Does not form indole nor reduce nitrates. Starch hydrolyzed but no acid produced on glucose, lactose, or sucrose. Aerobic. Produces 7.65 mgm. of ammonia and fixes 2.8 mgm. of nitrogen.

4. Gram-negative cocci 0.7 to 1.2 μ , occurring in clusters and pairs. Slow growth on ordinary laboratory media. Liquefies gelatin. Abundant tan growth on potato. Slow peptonization of milk with alkaline reactions. Does not produce indole nor reduce nitrates. Hydrolyzes starch but does not produce acid on glucose, sucrose, or lactose. Aerobic. Produces 6.46 mgm. of ammonia and fixes 4.2 mgm. of nitrogen in soil.

6B. Gram-positive rods 0.9 to 1.2 by 1.9 to 3.3 μ , occurring in chains. Motile by means of mono-trichious flagellum. Terminal spherical spores 0.5 to 0.7 μ . Grows readily on all ordinary laboratory media except potato. Abundant peach-colored growth on agar. Liquefies gelatin rapidly. Hydrolyzes starch. Produces indole. Reduces nitrates. Produces no acid on glucose, lactose, or sucrose. Aerobic. Produces 6.12 mgm. of ammonia and fixes 5.6 mgm. of nitrogen in soil.

6C. Gram-positive, motile rods 0.8 to 0.9 by 2 to 2.3 μ , occurring singly and in pairs. Central spores 1.0 to 1.8 μ . Rapid growth on all ordinary media except potato. Slowly liquefies gelatin. Does not produce indole nor reduce nitrates. Hydrolyzes starch. Produces acid on dextrose but not on lactose or sucrose. Aerobic. Produces 5.44 mgm. of ammonia and fixes 2.8 mgm. of nitrogen in soil.

7. Mold: Mycelium septate, straight with little branching. Conidia formed as in *Fusarium*; aerial mycelium formed, abundant growth on all ordinary laboratory media. Liquefies

gelatin. Hydrolyzes starch. Reduces nitrates to nitrites. Produces acid on glucose but not on lactose or sucrose. Aerobic. Produces 17 mgm. of ammonia from urea and 6.33 mgm. from peptone. Fixes 2.8 mgm. of nitrogen in soil.

10B. Gram-positive motile rods 0.3 to 0.6 by 1.4 to 3.6 μ . Abundant growth on all ordinary laboratory media. Rapidly liquefies gelatin. Abundant tan growth on potato. Indole formed. Reduces nitrates with the production of gas. Starch hydrolyzed. Aerobic. Produces 276 mgm. of ammonia from urea and 7.48 mgm. of ammonia from peptone. Fixes 1.4 mgm. of nitrogen in soil.

11A. Gram-negative, non-motile rods 0.5 to 0.6 by 2.4 to 6 μ occurring singly and in clusters. Grows well on ordinary media, does not liquefy gelatin. Slowly peptonizes milk with alkaline reaction. Produces indole, does not reduce nitrates, does not ammonify peptone. Fixes 7.0 mgm. of nitrogen in soil.

12A. Gram-positive, non-motile, non-spore-forming rod 0.6 to 0.8 by 1.5 μ , occurring singly and in clusters. Grows rapidly on agar and potatoes, slowly on gelatin and broth. Slowly peptonizes milk with the reduction of litmus. Produces indole. Does not reduce nitrates. Hydrolyzes starch, produces no acid on glucose, lactose, or sucrose. Aerobic. Produces 3 mgm. of ammonia from urea and 9.7 mgm. of ammonia from peptone. Does not fix nitrogen.

12B. Mold: Mycelium septate, straight with little branching. No aerial mycelium formed. Chlamydo-spores and conidia formed. Conidia oval in shape and connected in long chains. Rapid growth on ordinary laboratory media. Rapidly peptonizes milk first with acid, later turning alkaline. Blue-green, abundant growth on potato. Nitrates not reduced, starch hydrolyzed. Aerobic. Produces 4 mgm. of ammonia from urea and 167 mgm. of ammonia from peptone, does not fix nitrogen.

15A. Gram-positive, non-motile. Micrococci 0.8 to 1.1 μ in diameter. Rapid growth on agar. Slow growth on gelatin without liquefaction, produces no growth on potato. Produces indole, reduces nitrates, hydrolyzes starch, produces acid on glucose but not on sucrose nor lactose. Produces 4.6 mgm. of ammonia from peptone and fixes 4.2 mgm. of nitrogen in soil.

16B. Gram-positive, non-spore-forming rods 0.6 to 0.7 by 1.0 to 1.7 μ , occurring singly. Grows rapidly on all ordinary laboratory media and produces light yellow pigment on agar and potato. Liquefies gelatin. Slowly peptonizes milk with change in reaction. Reduces litmus. Produces indole. Reduces nitrates with the formation of gas. Hydrolyzes starch, produces acid on glucose and sucrose but not on lactose. Aerobic. Produces 8.5 mgm. of ammonia on peptone and fixes 7 mgm. of nitrogen in soil.

16C. Gram-positive, non-motile, non-spore-forming rods 0.8 to 0.9 by 1.8 to 2.7 μ , occurring singly, in pairs, and in short chains. Slowly liquefies gelatin. Rapid growth in other laboratory media. Potato abundant light cream tan growth. Produces indole. Does not reduce nitrates, hydrolyzes starch, produces acid on glucose but not on sucrose nor lactose. Aerobic. Very slowly ammonifies peptone.

16E. Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.6 by 0.7 to 0.9 μ , occurring singly and in clusters. Rapid growth on ordinary laboratory media. Creamish tan growth on potato. Liquefies gelatin. Produces indole. Does not reduce nitrates nor hydrolyze starch. Produces acid on glucose but not on sucrose or lactose. Aerobic. Slowly ammonifies peptone. Fixes 5.6 mgm. of nitrogen in soil.

17B. Gram-negative, non-motile, micrococci 0.8 to 1.0 μ . Rapid growth on agar. Slow growth without liquefaction on gelatin. No growth on potato. Hydrolyzes starch, reduces nitrates, and produces acid on glucose and lactose, but not on sucrose. Aerobic. Produces 13.8 mgm. of ammonia in peptone and fixes 5.6 mgm. of nitrogen in soil.

The morphological and physiological properties of the various organisms are summarized in table 1.

It is evident, from the results reported in table 1, that of the 17 organisms only 1 is able to ferment lactose, 3 sucrose, and 9 glucose. Not one produced gas. All but 3 liquefied gelatin. A majority of the organisms peptonized milk. The reaction in the majority of cases was alkaline.

With one exception, all organisms were aerobic, and this one was facultative anaerobic. There were 10 bacilli, 4 cocci, and 1 mold. Of the 16 cultures 6 formed spores, while only 4 were motile. Of the 16, 12 hydrolyzed starch at varying rates, 11 liquefied gelatin, 6 reduced nitrates to nitrites, 10 were

TABLE 1

Summary of principal morphological and physiological properties of organisms from leached synthetic alkali soil that will grow on nitrogen-free media

| CULTURE NUMBER | NITRATE REDUCTION | GELATIN LIQUEFACTION | HYDROLYSIS OF STARCH | MILK PEPTONIZED | INDOLE PRODUCTION | LITMUS MILK REACTION | ACTION ON GLUCOSE | ACTION ON LACTOSE | ACTION ON SUCROSE | SHAPE* | MOTILITY | GRAM REACTION | CHROMOGENESIS | SPORE FORMATION | AMMONIFICATION NH ₃ PRODUCED | N ₂ FIXED PER 100 GM. SOIL |
|----------------|-------------------|----------------------|----------------------|-----------------|-------------------|----------------------|-------------------|-------------------|-------------------|--------|----------|---------------|---------------|-----------------|---|---------------------------------------|
| | | | | | | | | | | | | | | | mgm. | mgm. |
| 1a | - | + | + | + | + | Alkaline | 0 | 0 | 0 | C | - | - | - | - | 6.3 | 1.4 |
| 1b | - | + | + | + | + | Alkaline | 0 | 0 | 0 | B | - | - | - | + | 7.7 | 2.8 |
| 4 | - | + | + | + | - | Alkaline | 0 | 0 | 0 | C | - | - | - | - | 6.5 | 4.2 |
| 6b | + | + | + | - | + | Acid | 0 | 0 | 0 | B | + | + | + | + | 6.1 | 5.6 |
| 6c | - | + | + | - | - | Acid | + | 0 | 0 | B | + | + | + | + | 5.4 | 2.8 |
| 7 | + | + | - | + | - | Acid and alkaline | + | 0 | 0 | F | - | + | + | + | 6.6 | 2.8 |
| 9a | - | + | + | + | - | Acid | + | 0 | + | B | + | + | - | + | 9.4 | 0.0 |
| 10b | + | + | - | + | + | Alkaline | 0 | 0 | 0 | B | + | + | + | + | 7.5 | 1.4 |
| 11a | - | - | - | + | + | Alkaline | + | 0 | 0 | B | - | - | - | - | 0.7 | 7.0 |
| 12a | - | - | - | + | + | Alkaline | 0 | 0 | 0 | B | - | + | - | - | 9.7 | 0.0 |
| 12b | - | + | + | + | - | Alkaline and acid | 0 | 0 | + | F | - | + | - | - | 16.7 | 0.0 |
| 15a | + | - | + | - | + | Alkaline | + | 0 | 0 | C | - | + | + | - | 4.6 | 4.2 |
| 16b | + | + | + | + | + | 0 | + | 0 | + | B | - | + | + | - | 8.5 | 7.0 |
| 16c | - | + | + | + | + | 0 | + | 0 | 0 | B | - | + | + | - | 0.5 | |
| 16e | - | + | - | + | + | Acid | + | 0 | 0 | B | - | + | - | - | 0.2 | 5.6 |
| 17b | + | - | + | - | - | 0 | + | + | 0 | C | - | - | + | - | 13.8 | 5.6 |

* C, cocci; B, rods; F, filamentous.

gram-positive, and 9 produced indol. None of the organisms decomposed cellulose, 5 fermented urea, all but 3 produced ammonia, and 7 fixed appreciable quantities of nitrogen.

Of the 15 organisms tested, 12 fixed nitrogen when grown in soil but up to date no appreciable nitrogen fixation has been obtained in synthetic media. The gains in nitrogen which have been obtained in soils vary from 1.4 to 7 mgm. per 100 gm. of soil. Seven of the soils showed gains of over 4 mgm. of nitrogen. All but 3 of the organisms ammonified peptone, 1 produced 16.7 mgm. Four organisms ammonified urea, 1 producing 276 mgm.

SUMMARY

A study was made of the microorganisms which developed on Ashby agar from a leached synthetic alkali soil. Sixteen organisms were obtained in pure culture. Of this number 12 fixed nitrogen. The nitrogen-fixing ability of these varied from 1.4 mgm. to 7 mgm. Seven fixed over 4 mgm. in the 5-week incubation period on sterile soil to which 2.5 per cent mannitol had been added. Of the 16, 12 produced over 5 mgm. of ammonia in 4 days on a 1 per cent peptone solution. It was found that 5 of the organisms decomposed urea, whereas not one decomposed cellulose.

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COMPOSITION AND NITRIFICATION STUDIES ON CROTALARIA STRIATA¹

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Since the work of Krause and Kraybill (5) and others on the difference of plant composition at different growth stages has established a relationship between such plant compositions and the various plant functions, many horticultural and agronomic practices are being conducted with these findings as a basis. This variation in plant composition at different growth stages has not only been correlated with plant functions, but also, with the rate of the decomposition of plant material when incorporated with the soil. Thus an accurate analysis of plant materials to be incorporated with the soil, together with suitable observations on the biological processes which take place therein, should give a more accurate picture of the problem of the accumulation and utilization of organic matter in the soil, and lead to an adequate explanation of the numerous green manure practices.

The decomposition of plant materials in the soil has been studied from several angles. Thus, the end-products of the plant constituents have been measured. Carbon dioxide and nitrate nitrogen have been used as a measure of the decomposition of plant constituents. The accumulation of so-called humus has also been measured. Finally, the response of a crop following the incorporation of organic matter with the soil has been utilized as a measure of the decomposition of organic plant constituents in the soil. Without an accurate knowledge of the composition of the plant material incorporated, it is doubtful whether any or a combination of these various methods would be of very great value in an interpretation of the decomposition of plant material in the soil. Thus, the composition of the plant material largely controls the end-products which are obtained and the ultimate effects on plant growth and the accumulation of organic residues in the soil. The processes taking place, and consequently the end-products, naturally change with the environment of the soil; particularly in regard to oxygen supply, but the composition of the plant material must necessarily control the formation of such products.

The carbon-nitrogen ratio of soil organic matter and of plant and animal

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materials incorporated with soil has been used to determine what might be expected in end-products from their decomposition. Although the nitrogen-carbon ratio of decomposing organic matter gives in general a fair idea of the end-products to be expected, still the exceptions show that a more detailed analysis of materials is necessary to understand the processes which take place and the ultimate end-products which may be expected from a given material. Thus, a more detailed analysis of plant materials giving the proportions of the nitrogen-bearing compounds (or forms of nitrogen) to those bearing no nitrogen will give a better conception of the processes taking place in the soil when such materials are incorporated therewith. The energy relationships may certainly be better ascertained. With these relationships better understood, the selection and utilization of many of our green manuring crops should be better realized.

Among the more recently introduced green manure plants in the southeastern states, the *Crotalaria striata* stands out as one of the most promising, especially for the light sandy soils of Florida. The general characteristics and growth habits of this plant and its value as a green manure measured by crop response as given by Stokes (7) necessitated a more detailed study of its varying composition at different growth stages and the effect of these differences in composition upon its decomposition in the soil as measured by nitrate formation and accumulation.

COMPOSITION OF CROTALARIA PLANT

Preparation of Material

The *Crotalaria* plants for composition studies were grown under greenhouse conditions in 2-gallon jars with 10 kilos of air-dried Norfolk sand kept at an optimum moisture content of 50 per cent of its water-holding capacity. Each jar contained five plants. The plants from a sufficient number of jars, generally six to eight, were dug at different growth stages as follows: when young and succulent; in an advanced vegetative growth stage; when in bloom; in seed stage; in late fall or semi-dormant condition; and prepared for analysis. The roots were separated from the tops and washed in cold running water to remove adhering soil particles. The green weights of both tops and roots were recorded. They were then cut into small pieces (one-quarter to one-eighth inch in length) and 250 gm. of each placed in wide-mouthed bottles. Enough concentrated hot 95 per cent ethyl alcohol was added to each to insure a concentration of 75 per cent. The bottles were then stoppered with corks covered with tinfoil and placed in a dry room for analysis later. Duplicate 15-gm. samples of each, tops and roots, were placed in an electric oven at 105°C. to determine the percentage of dry matter. All results were computed on a dry weight basis except the percentage of dry matter.

Methods of plant analysis

Easily hydrolyzed carbohydrates. Three-gram samples in triplicate were used for analysis and results computed on a dry weight basis. The reducing

power of the various carbohydrate extractions was determined by the Schaffer-Hartman (6) volumetric method for sugar analysis, after appropriate hydrolysis, where necessary. All carbohydrate percentages were expressed in terms of glucose.

Sugars. The alcohol extract from the preserved material, after standing for several weeks, was filtered into a beaker. The preserved material was placed upon the filter paper and washed with a volume of warm 95 per cent alcohol equal to the volume of the extract. The filtrate was then evaporated on a steam bath to a syrup. The residue was then taken up with distilled water, clarified with neutral lead acetate, and filtered through dry folded filter paper. A sufficient quantity of a mixture of nine parts of anhydrous Na_2SO_4 and one part of Na_2CO_3 was added to the filtrate to remove any excess of lead. After the solution was filtered through fine filter paper, it was made up to 500 cc. Fifty-cubic centimeter aliquots without hydrolysis were used to determine reducing sugars. A 50-cc. aliquot was diluted to 90 cc., 10 cc. of concentrate HCl (sp. gr. 1.12) added, and hydrolyzed on a sand bath for one hour. The reducing power was then determined for total sugars after the solution was neutralized with 40 per cent NaOH as for reducing sugars. The residue on the filter paper was dried in a ventilated oven at 70°C . This dried material was then ground in a Quaker mill and pulverized in a Dreef (11) mill until fine enough to pass through a 60-mesh sieve. It was then preserved in tightly stoppered bottles for analysis later. Three-gram samples of this dried material were used for carbohydrate analysis. After being redried in a Cenco vacuum oven at 60°C . the total and reducing sugars were determined on the entire residue from the 3-gm. samples. These sugars, after the removal of the alcohol extract, were then computed and added to the weights of similar sugars found in the alcoholic extract. The percentage of each sugar was then computed on the total dry material in the preserved sample.

Soluble starches and dextrins. After the sugars were extracted from the sample, 50 cc. of water was added to the residue in a beaker and allowed to stand for 12 hours. This mixture was then filtered and the residue washed until the filtrate attained a volume of 90 cc. Ten cubic centimeters concentrated HCl (sp. gr. 1.12) was added to the filtrate, which was then hydrolyzed on a sand bath under a reflux condenser for two and one-half hours. The solution was then cooled, neutralized with 40 per cent NaOH , and made up to 250 cc. Fifty-cubic centimeters aliquots were used to determine the reducing power.

Starch. The residue, after the extraction of soluble starches and dextrins, was washed into a beaker and the volume made up to 50 cc. The mixture was then heated to boiling to change the starch to a paste. The temperature was lowered to 38°C ., 10 cc. of fresh saliva added, and the mixture digested at this temperature for one-half hour. At the end of this digestion period, the mixture was again heated to boiling, filtered, and the residue thoroughly washed with hot water until the filtrate attained a volume of 90 cc. Next 10 cc. of

concentrated HCl (sp. 1.12) was added to the filtrate, which was hydrolyzed on a sand bath under a reflux condenser for two and one-half hours. The solution was then cooled, neutralized with 40 per cent NaOH, and made up to a 250 cc. volume. To determine the reducing power, 50-cc. aliquots were used.

Hemicelluloses. To remove hemicelluloses, the final residue (after removal of starches) was washed into an Erlenmeyer flask, made up to 90 cc. volume, 10 cc. of concentrated HCl (sp. 1.12) added, and hydrolyzed on a sand bath under a reflux condenser for two and one-half hours. The mixture in the flask was then filtered and washed with hot water. The filtrate was next neutralized, clarified, delead, and made up to a 500 cc. volume. To determine the reducing power, 50 cc. aliquots were used.

Cellulose. The residue (after the extraction of hemicelluloses) was boiled in 100 cc. of 1 per cent NaOH under a reflux condenser for one-half hour. This mixture was then filtered on a Hirsch funnel with suction and washed several times with distilled water. After the water was extracted with suction, the material was exposed to chlorine gas for 20 minutes. After chlorination, the fiber was washed with sulfurous acid and then boiled in 100 cc. of 2 per cent Na_2SO_3 , filtered, washed twice with hot water, and then again removed to a beaker and chlorinated for five minutes. The washing with sulfurous acid and boiling with 2 per cent Na_2SO_3 was repeated until the acid remained practically colorless when added to the tissue. The fiber was then washed well four to six times with hot water, followed by washing with 5 per cent acetic acid. The acetic acid was washed out with hot water. The fiber was removed to an alundum crucible, washed with alcohol and finally with ether. The percentage of cellulose was determined by loss on ignition.

Lignin. A 3-gm. sample in triplicate was extracted with ether (alcohol free anhydrous) to remove fats, waxes and resins, and then transferred to a beaker. Fifteen cubic centimeters of 73 per cent H_2SO_4 was added and the mixture allowed to stand for 16 hours. The mixture was then diluted with water to a 3 per cent solution of H_2SO_4 and boiled for two hours to coagulate the residue. The mixture was then filtered through an alundum crucible and washed well with hot water. The percentage of lignin was determined by loss on ignition.

Nitrogen. The various forms of nitrogen in the roots, tops, leaves, and stems of the plants were determined on the green material and the percentage of each was computed on a dry weight basis. Fifty grams of the green material of each plant part were triturated in a large mortar with quartz sand previously washed free of foreign material. About 5 cc. of ether was added to promote plasmolysis, and water was added as required to give a proper consistency for trituration. When the material was finely ground (so as to break up the plant cells), it was extracted with distilled water through four thicknesses of cheesecloth, and the extract was then filtered through a moist paper pulp filter in a Buchner funnel with slight suction to a volume of 1900 cc. The filtrate was

then made up to a volume of 2000 cc. with distilled water drawn through the paper pulp.

Forms of extracted nitrogen. One hundred cubic centimeters of the extract was used to determine total water-extracted nitrogen by the Kjeldahl method modified to include nitrate nitrogen. Two hundred and fifty cubic centimeters aliquots were brought to boiling and the coagulable proteins precipitated with 10 per cent acetic acid. After the precipitate was filtered and washed with hot water, it was transferred with the filter paper to a Kjeldahl flask, and total nitrogen determined therein by the Kjeldahl method. Aliquots from the filtrate were used to determine amino acid and nitrate nitrogen. Amino acid nitrogen was determined by the Van Slyke method. Nitrate nitrogen was determined by the DeVarda method (1). The percentage of total extracted nitrogen minus that of the coagulable protein is here termed "water-soluble nitrogen."

Unextracted nitrogen. Unextracted nitrogen was obtained by subtracting the percentage of total water-extracted nitrogen from the percentage of total nitrogen.

Total protein nitrogen. The percentage of coagulable protein nitrogen was added to that of the unextracted nitrogen and termed total "protein nitrogen."

Total nitrogen. Total nitrogen was determined on 3-gm. samples of the originally dried material by the Kjeldahl method modified to include nitrate nitrogen.

Experimental data

The data tabulated in tables 1 and 2 show a striking variation in the percentages of dry matter, the various carbohydrate compounds, and the different forms of nitrogen or nitrogen compounds from one growth stage to another during the season. Total sugars and the easily hydrolyzable polysaccharides; namely, dextrans, starches, and hemicelluloses, are comparatively high in percentage during the early succulent growth of the plants but show a marked decrease in percentage when the plants are in a more advanced vegetative growth stage. This may be accounted for by the rapid growth of the plants and the increased demand by the plant for these materials as a result of its increased respiration and anabolic processes. These compounds appear to be more constant in percentage in the different plant parts through the successive growth stages.

The percentages of cellulose in the tops and roots and the whole plant show a gradual increase from 15.86, 13.51, and 15.50, respectively, in the early succulent growth stage to 27.37, 31.37, and 28.55 for these respective plant parts in the late fall or dormant growth stage. A comparison of the lignin in the roots, tops, and the whole plant from one growth stage to another does not show much variation in percentage, but remains more or less constant. The dry matter in the tops, roots, and the whole plant shows a gradual increase in percentage from

TABLE 1

Percentage of dry matter, sugars, polysaccharides, total easily hydrolyzable carbohydrates, cellulose and lignin in tops, roots, and whole plant of *Crotalaria striata* at different stages of growth during season of 1926

All percentages calculated on a dry weight basis except dry matter

| STAGE OF GROWTH | DRY MATTER | | | REDUCING SUGARS | | | TOTAL SUGARS | | | TOTAL POLYSACCHARIDES | | |
|-------------------------------|---------------------------------------|----------|-------------|-----------------|----------|-------------|--------------|----------|-------------|-----------------------|----------|-------------|
| | Tops | Roots | Whole plant | Tops | Roots | Whole plant | Tops | Roots | Whole plant | Tops | Roots | Whole plant |
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| Succulent..... | 13.785 | 9.510 | 12.920 | 3.922 | 3.080 | 3.385 | 11.670 | 6.730 | 10.934 | 9.964 | 15.225 | 10.456 |
| Vegetative..... | 15.311 | 11.430 | 14.443 | 1.510 | 2.313 | 1.356 | 5.884 | 3.024 | 3.731 | 8.769 | 7.636 | 8.568 |
| Early bloom..... | 20.700 | 15.450 | 20.047 | 0.790 | 1.080 | 0.817 | 3.924 | 2.896 | 3.825 | 15.978 | 14.508 | 15.836 |
| Bloom and seed pod..... | 29.900 | 23.887 | 28.839 | 1.985 | 1.470 | 1.893 | 2.211 | 2.808 | 2.392 | 15.900 | 11.288 | 15.082 |
| Late seed pod or dormant..... | 42.437 | 26.185 | 36.089 | 1.557 | 1.856 | 1.641 | 3.189 | 3.014 | 3.153 | 17.844 | 12.343 | 16.279 |
| | TOTAL EASILY HYDROLYZED CARBOHYDRATES | | | CELLULOSE | | | LIGNIN | | | | | |
| Succulent..... | 21.634 | 21.955 | 21.390 | 15.860 | 13.510 | 15.504 | | | | | | |
| Vegetative..... | 14.653 | 10.660 | 12.299 | 19.300 | 22.543 | 22.130 | 18.600 | 22.700 | 19.325 | | | |
| Early bloom..... | 19.902 | 17.404 | 19.661 | 21.498 | 25.081 | 21.841 | 22.560 | 25.960 | 22.879 | | | |
| Bloom and seed pod..... | 18.111 | 14.096 | 17.474 | 26.255 | 27.630 | 26.498 | 21.430 | 21.100 | 20.617 | | | |
| Late seed pod or dormant..... | 21.033 | 15.357 | 19.432 | 27.375 | 31.373 | 28.551 | 20.560 | 20.100 | 20.429 | | | |

TABLE 2
Percentage of dry matter and different forms of nitrogen in tops, roots, and whole plant of Crotalaria striata at different stages of growth during 1926
 All percentages except dry matter calculated on a dry weight basis

| STAGE OF GROWTH | DRY MATTER | | | WATER-EXTRACTED NITROGEN | | | NON-WATER-EXTRACTED NITROGEN | | | COAGULABLE PROTEIN NITROGEN | | |
|-------------------------------|------------------------|--------|-------------|--------------------------|-------|-------------|------------------------------|-------|-------------|-----------------------------|-------|-------------|
| | Tops | Roots | Whole plant | Tops | Roots | Whole plant | Tops | Roots | Whole plant | Tops | Roots | Whole plant |
| | | | | | | | | | | | | |
| Succulent..... | 13.785 | 9.510 | 12.920 | 1.438 | 1.567 | 1.449 | 2.042 | 0.536 | 1.812 | 0.687 | 0.770 | 0.689 |
| Late vegetative..... | 15.311 | 11.430 | 14.443 | 1.282 | 1.072 | 1.238 | 1.521 | 0.461 | 1.339 | 0.528 | 0.447 | 0.512 |
| Early bloom..... | 20.700 | 15.450 | 20.047 | 0.747 | 0.997 | 0.770 | 1.484 | 0.203 | 1.361 | 0.274 | 0.357 | 0.281 |
| Bloom and seed pod..... | 29.900 | 23.887 | 28.839 | 0.782 | 0.425 | 0.742 | 0.824 | 0.625 | 0.784 | 0.436 | 0.379 | 0.423 |
| Late seed pod or dormant..... | 42.473 | 26.185 | 36.089 | 0.471 | 0.412 | 0.454 | 0.652 | 0.970 | 0.742 | 0.245 | 0.311 | 0.263 |
| | TOTAL PROTEIN NITROGEN | | | WATER-SOLUBLE NITROGEN | | | TOTAL NITROGEN | | | | | |
| Succulent..... | 2.729 | 1.306 | 2.501 | 0.751 | 0.797 | 0.760 | 3.480 | 2.103 | 3.261 | | | |
| Late vegetative..... | 2.049 | 0.908 | 1.851 | 0.754 | 0.625 | 0.726 | 2.403 | 1.533 | 2.577 | | | |
| Early bloom..... | 1.758 | 0.560 | 1.642 | 0.473 | 0.640 | 0.478 | 2.231 | 1.200 | 2.131 | | | |
| Bloom and seed pod..... | 1.260 | 1.004 | 1.207 | 0.346 | 0.046 | 0.289 | 1.606 | 1.050 | 1.496 | | | |
| Late seed pod or dormant..... | 0.897 | 1.281 | 1.005 | 0.226 | 0.101 | 0.191 | 1.123 | 1.382 | 1.196 | | | |

the early succulent stage to the late fall or dormant stage. On a quantity basis, this increase in dry matter would naturally show a marked increase in actual plant material and quantities of nitrogen and carbohydrate compounds from one growth stage to another.

The tops of the plants and the whole plant show similar variations in percentages of the different forms of nitrogen. The total nitrogen, total water-extracted nitrogen, total protein nitrogen, and unextracted nitrogen show a gradual decrease in percentage from the early succulent through the successive growth stages up to dormancy. A similar trend in percentage is shown in the roots except in the case of the unextracted, total, and total protein nitrogen, which show a slight increase in percentage in the two later growth stages (table 2).

This variation in the percentages of the various carbohydrate compounds and different forms of nitrogen or nitrogen compounds from one growth stage to another naturally creates a striking difference in the relation or ratio between these compounds at these different growth stages. The higher percentage of these various nitrogen forms in the early growth stages produces a narrow carbohydrate-nitrogen ratio, whereas a decrease in the percentage of these different forms of nitrogen compared with the higher percentages of some of the higher carbohydrates in the later growth stages produces a wide carbohydrate-nitrogen ratio during the later growth stages.

This widening in the ratio between nitrogen and carbohydrates is most pronounced when these nitrogen percentages are compared with the percentages of cellulose from one growth stage to another. A gradual decrease in these nitrogen forms and the increase in the percentage of carbohydrates makes the widening of this carbohydrate-nitrogen ratio from one stage to another very pronounced.

COMPOSITION OF *Crotalaria striata* IN RELATIONSHIP TO ITS DECOMPOSITION IN THE SOIL

A marked difference was noted in the percentages of different carbohydrate compounds and various forms of nitrogen or nitrogen compounds in the foregoing study of the *Crotalaria* plant. Likewise, a striking difference in the ratio between the carbohydrate compounds and the different forms of nitrogen or nitrogen compounds was shown. These variations in plant composition indicate that there would exist a difference in the formation and accumulation of the different end-products of decomposition when these materials are incorporated with the soil. For the purpose of ascertaining the possible effects of this variation in the composition of the *Crotalaria* plant at different growth stages on the formation and accumulation of end-products in the soil, another series of plants was analyzed at different growth stages and decomposition studies were made.

Preparation of material

The plants in this series were grown under field conditions. Sufficient plants were dug at four different growth stages as follows:

1. When the plants were young and succulent.
2. When the first flowers appeared (early reproductive stage).
3. In the advanced seed pod stage.
4. In the late fall or early winter condition (leaves killed by frost).

The plants were taken from the field to the laboratory and separated into the leaves, stems, and roots. Seed pods in the later stages were included with the leaves. These different plant parts were cut into small pieces (one-quarter to one-eighth inch) and dried in a ventilated oven at 70°C. The materials were then ground in a Quaker mill, pulverized in a Dreef mill, and preserved in tightly stoppered bottles for analysis.

Methods of analysis

Sugars. The dried samples were redried in a Cenco vacuum oven at 60°C. The reducing power was then determined on 3-gm. samples, as described in the preceding, after extracting with ether (anhydrous alcohol free) to remove fats, waxes, and resins.

Other carbohydrates. The polysaccharides and other carbohydrates were determined as previously described.

Nitrogen. All forms of nitrogen were determined as described.

Soils studies

The green plant parts were passed through a power food chopper, and 100 gm. of each finely ground plant part were incorporated with 20 pounds of air-dried Norfolk fine sandy soil; 100 gm. of the entire green plant made up of the different plant parts combined in the growth proportions were likewise incorporated. The soils were maintained at an optimum moisture content and incubated under greenhouse conditions.

This procedure was repeated for the plants when they reached the different growth stages mentioned.

Fifty-gram samples of soil were carefully weighed from the duplicate pots in which the soil was composted with the different plant parts and with the whole plant. The nitrate content of the soil was determined by making a 1 to 5 water extract of the soil immediately after sampling and using the phenoldisulfonic acid method. These samples were taken every two weeks after incubation was started. According to Waksman (8), the accumulation of nitrates in the soil may be used as an indication of the decomposition processes or biological activity taking place in the soil. From the nitrate content of the soil in the different pots, the total nitrogen present as nitrate was calculated for each sampling. The amount of nitrogen as nitrate in the soil receiving no additions was subtracted from the amount present in the soil of the green

manured pots and the remainders calculated as percentages of the total nitrogen added in the green manure.

This procedure makes possible a direct comparison between the degree of nitrate accumulation from the different plant parts and from the whole plant when incorporated with the soil at different growth stages, regardless of the fact that the actual quantities of dry matter and nitrogen varied with the plant part and the stage of growth.

A comparison of nitrate accumulation from different plant parts

A comparison of the carbohydrate percentages as given in table 3 with the nitrogen percentages given in table 4, shows striking variations in the ratio between such carbohydrate and nitrogen compounds in the *Crotalaria* plant at different growth stages. This marked variation can be more easily observed by an examination of figures 1, 2, 3, and 4, which represent graphically such carbohydrate and nitrogen percentages in the whole *Crotalaria* plant and its separate parts at different growth stages. The term "carbohydrates" as used on these graphs includes sugars, starches, dextrans, and hemicelluloses or the "easily hydrolyzed carbohydrates" as given in the preceding tables. The differences in the amounts of nitrate accumulation, expressed as the percentage of the total nitrogen added, at the different periods, are also given. A comparison of the differences in the composition of the plant and its parts with the percentages of the total nitrogen added present as nitrates at definite periods after the incorporation of the same with the soil, shows a marked correlation.

The degree of nitrate accumulation from the *Crotalaria* plant and its parts when incorporated with the soil is shown to be in the ascending order; roots, stems, complete plant, and leaves for all the different growth stages. In the early stages of decomposition, the stems and roots show a negative percentage of nitrogen nitrified, which indicates a utilization of the nitrogen (nitrates) of the soil in the decomposition of these plant parts. Conversely, there is a progressive increase in the percentage of nitrogen of the complete plant and of the leaves nitrified for each of these growth stages. These differences in the degree of nitrate accumulation in the soil from the plant and its parts are correlated with differences in the composition of these materials.

As indicated in table 5, the averaged ratios of the total nitrogen to cellulose in the leaves, complete plants, stems, and roots for all the growth stages are 2.0, 7.5, 17.2 and 21.9, respectively. Likewise the ratios of the nitrogen to easily hydrolyzable carbohydrates for the above mentioned materials are 4.5, 9.5, 18.6, 20.5, respectively. Somewhat similar ratios may be noted between other forms of nitrogen and the cellulose and easily hydrolyzable carbohydrates, but the ratios given in table 5 correlate more perfectly with the accumulation of nitrates in the soil after the incorporation of these materials. These ratios between the total nitrogen and the cellulose and easily hydrolyzable carbohydrates in the plant materials correlate with the degree of nitrate accumulation

TABLE 3

Percentage of dry matter, ether extract, easily hydrolyzable carbohydrates, cellulose, and lignin in roots, stems, leaves, and the whole plant of *Crotalaria striata* at different growth stages during growing season of 1927

(All percentages calculated on dry weight basis except dry matter. All easily hydrolyzed carbohydrates given in terms of glucose)

| STAGE OF GROWTH | DRY MATTER | | | | | ETHER EXTRACT | | | | | REDUCING SUGARS | | | | | TOTAL SUGARS | | | | |
|---------------------------|-----------------------|----------|----------|----------|-------------|--------------------------------|----------|----------|----------|-------------|-----------------|----------|----------|----------|-------------|--------------|----------|----------|----------|-------------|
| | Leaves | | Stems | | Whole plant | Leaves | | Stems | | Whole plant | Leaves | | Stems | | Whole plant | Leaves | | Stems | | Whole plant |
| | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | |
| Succulent..... | 16.800 | 16.000 | 18.600 | 16.656 | | 6.655 | 1.650 | 1.180 | 4.026 | | 2.516 | 2.766 | 1.333 | 1.262 | | 7.216 | 4.066 | 3.033 | 5.448 | |
| Bloom..... | 19.200 | 22.600 | 23.200 | 21.160 | | 6.143 | 0.846 | 1.016 | 3.014 | | 1.683 | 1.750 | 0.996 | 1.615 | | 6.800 | 2.916 | 3.033 | 4.504 | |
| Seed pod..... | 32.400 | 38.800 | 37.800 | 35.609 | | 4.055 | 1.273 | 2.480 | 2.605 | | 0.966 | 1.683 | 1.200 | 1.325 | | 3.283 | 2.866 | 4.016 | 2.977 | |
| Late fall or dormant..... | 72.000 | 70.800 | 53.500 | 67.058 | | 1.036 | 0.866 | 0.766 | 0.879 | | 2.000 | 0.333 | 0.800 | 0.730 | | 2.816 | 2.000 | 2.273 | 2.203 | |
| | TOTAL POLYSACCHARIDES | | | | | TOTAL HYDROLYZED CARBOHYDRATES | | | | | CELLULOSE | | | | | LIGNIN | | | | |
| Succulent..... | 16.882 | 16.746 | 18.732 | 17.041 | 24.098 | 20.812 | 21.765 | 22.489 | | | 7.424 | 22.730 | 24.566 | 15.643 | | 8.619 | 18.015 | 20.198 | 13.786 | |
| Bloom..... | 13.748 | 19.450 | 18.365 | 16.987 | 20.548 | 22.366 | 21.398 | 21.491 | | | 8.320 | 20.686 | 19.609 | 15.526 | | 7.891 | 21.440 | 22.111 | 16.049 | |
| Seed pod..... | 17.949 | 23.332 | 21.082 | 20.761 | 21.232 | 26.198 | 25.098 | 23.738 | | | 12.163 | 21.066 | 22.766 | 17.272 | | 17.233 | 22.200 | 22.177 | 20.141 | |
| Late fall or dormant..... | 15.182 | 20.235 | 19.382 | 19.130 | 17.998 | 22.235 | 21.655 | 21.333 | | | 8.855 | 21.650 | 29.466 | 20.682 | | 18.200 | 27.975 | 22.425 | 25.122 | |

TABLE 4

*Percentage of dry matter, different forms of nitrogen and total nitrogen in leaves, stems, roots, and whole plant of *Crotalaria striata* at different stages of growth during season of 1927*

(All nitrogen percentages calculated on a dry weight basis)

| STAGE OF GROWTH | DRY MATTER | | | | | WATER-EXTRACTED NITROGEN | | | | | NON-WATER-EXTRACTABLE NITROGEN | | | | | COAGULABLE PROTEIN NITROGEN | | | | |
|---------------------------|------------------------|----------|----------|-------------|--|--------------------------|----------|----------|-------------|--|--------------------------------|----------|----------|-------------|--|-----------------------------|----------|----------|-------------|--|
| | Leaves | Stems | Roots | Whole plant | | Leaves | Stems | Roots | Whole plant | | Leaves | Stems | Roots | Whole plant | | Leaves | Stems | Roots | Whole plant | |
| | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | | per cent | per cent | per cent | per cent | |
| Succulent..... | 16.800 | 16.000 | 18.600 | 16.656 | | 1.666 | 0.675 | 1.010 | 1.186 | | 3.254 | 0.885 | 0.485 | 1.968 | | 0.205 | 0.260 | 0.387 | 0.248 | |
| Bloom..... | 19.200 | 22.600 | 23.200 | 21.160 | | 1.416 | 0.743 | 0.741 | 1.020 | | 4.359 | 0.682 | 0.409 | 1.732 | | 0.241 | 0.085 | 0.165 | 0.159 | |
| Seed pod..... | 32.400 | 38.800 | 37.800 | 35.609 | | 0.802 | 0.432 | 0.568 | 0.607 | | 2.921 | 0.628 | 0.325 | 1.616 | | 0.049 | 0.021 | 0.025 | 0.033 | |
| Late fall or dormant..... | 72.000 | 70.800 | 53.500 | 67.058 | | 1.022 | 0.446 | 0.254 | 0.518 | | 3.803 | 0.624 | 0.771 | 1.248 | | 0.072 | 0.018 | 0.009 | 0.026 | |
| | TOTAL PROTEIN NITROGEN | | | | | WATER-SOLUBLE NITROGEN | | | | | NITROGEN OF AMINO ACIDS | | | | | TOTAL NITROGEN | | | | |
| Succulent..... | 3.459 | 1.145 | 0.872 | 2.216 | | 1.461 | 0.415 | 0.623 | 0.938 | | 0.223 | 0.241 | 0.180 | 0.224 | | 4.920 | 1.560 | 1.495 | 3.154 | |
| Bloom..... | 4.600 | 0.767 | 0.574 | 2.265 | | 1.175 | 0.658 | 0.576 | 0.861 | | 0.140 | 0.109 | 0.087 | 0.117 | | 5.775 | 1.425 | 1.150 | 2.752 | |
| Seed pod..... | 2.970 | 0.649 | 0.350 | 1.648 | | 0.753 | 0.411 | 0.543 | 0.564 | | 0.163 | 0.142 | 0.221 | 0.157 | | 3.723 | 1.060 | 0.893 | 2.223 | |
| Late fall or dormant..... | 3.875 | 0.642 | 0.780 | 1.274 | | 0.950 | 0.428 | 0.245 | 0.492 | | 0.292 | 0.155 | 0.079 | 0.167 | | 4.825 | 1.070 | 1.025 | 1.766 | |

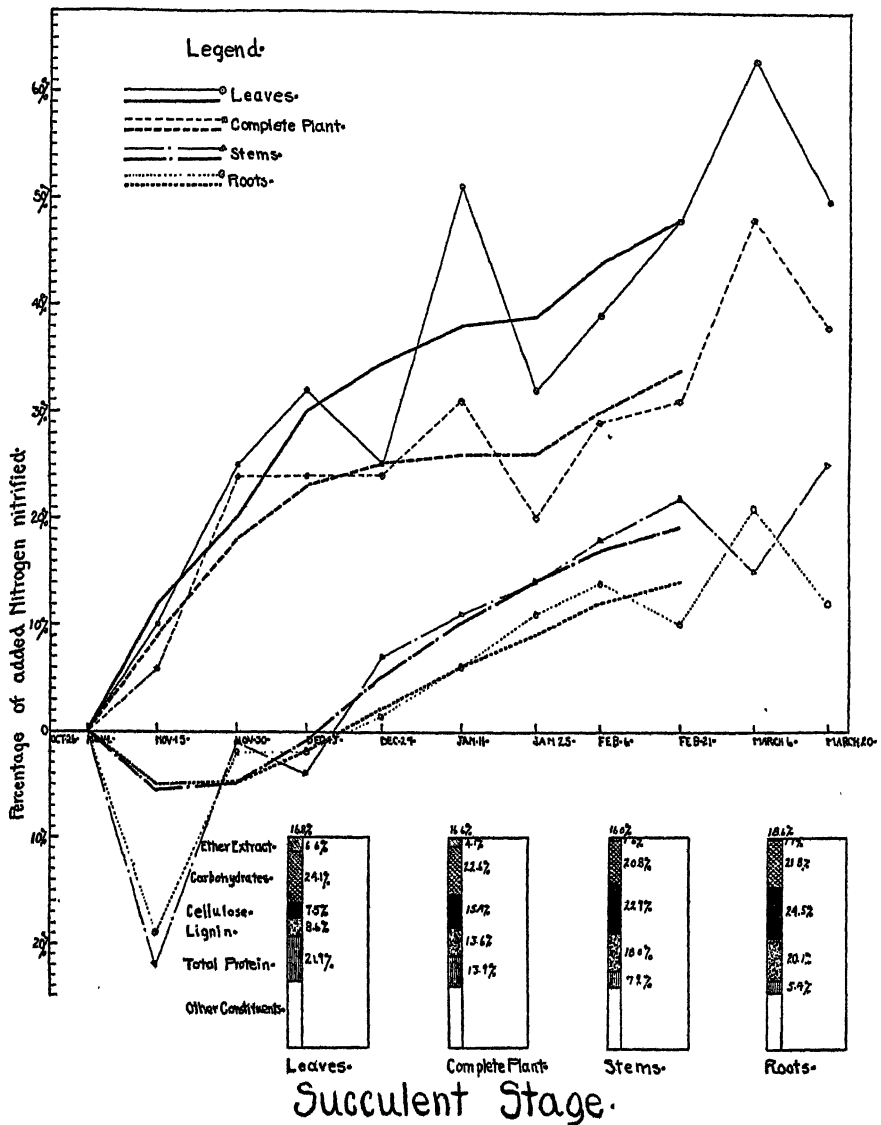


FIG. 1. PERCENTAGES OF NITROGEN NITRIFIED FROM THE *succulent* CROTALARIA PLANT AND ITS SEPARATE PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL, AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND ITS PARTS

in the soil: the narrow ratio in the leaves, giving the most rapid accumulation of nitrate, contrasted with the slower accumulation of nitrates for the soil, containing stems or roots with a wide ratio, in which an actual nitrogen deficit occurred.

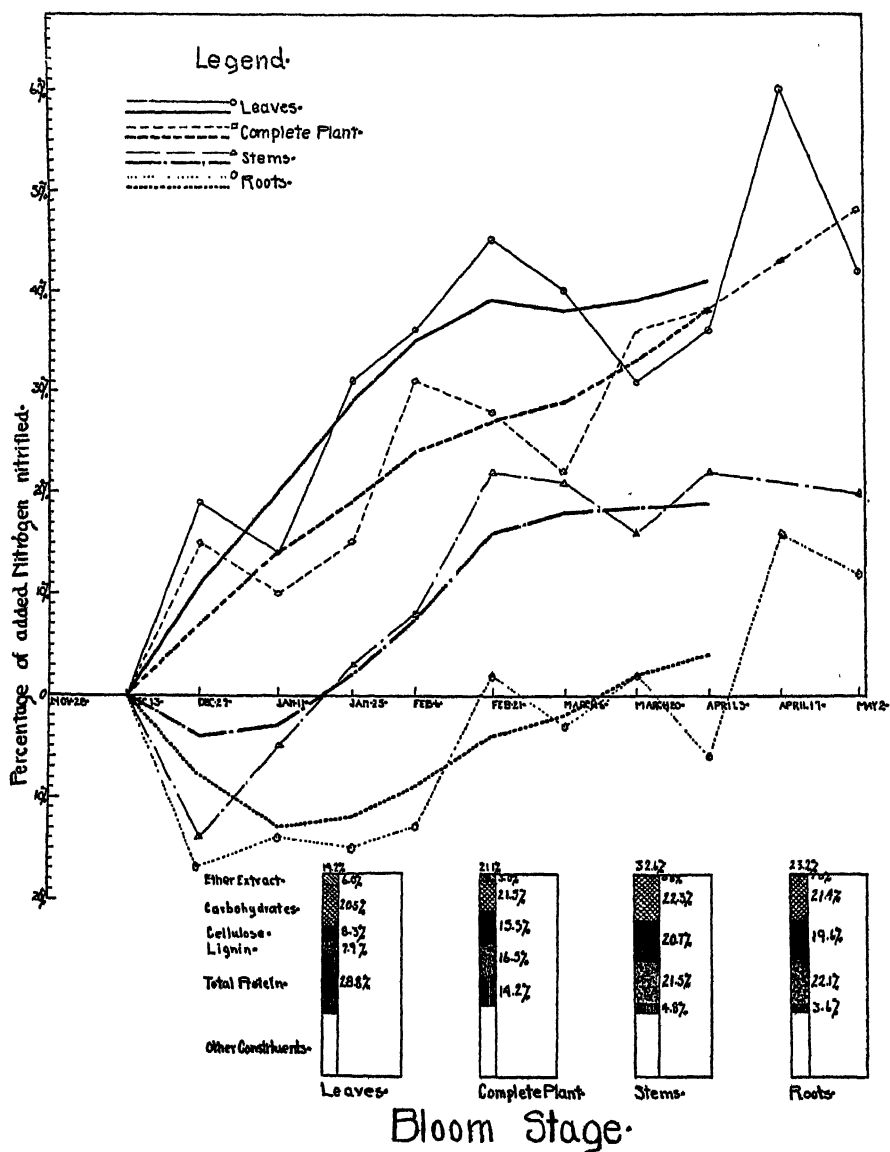


FIG. 2. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN THE EARLY bloom stage OF GROWTH

These results are in keeping with the findings of Waksman and Tenney (9) who have made a study of the decomposition of the rye plant at different stages of growth, using CO_2 evolution as a measure of decomposition. These authors

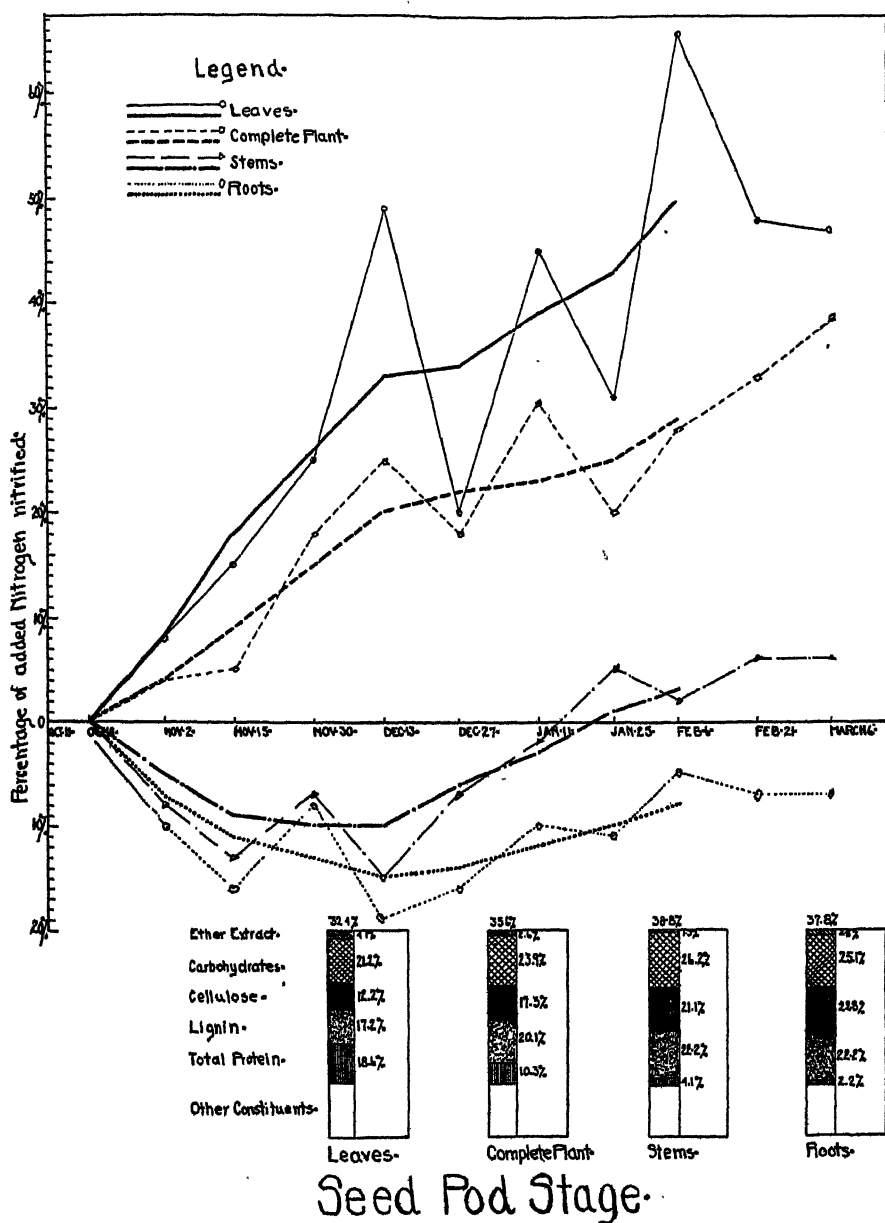


FIG. 3. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN THE *seed pod stage* OF GROWTH

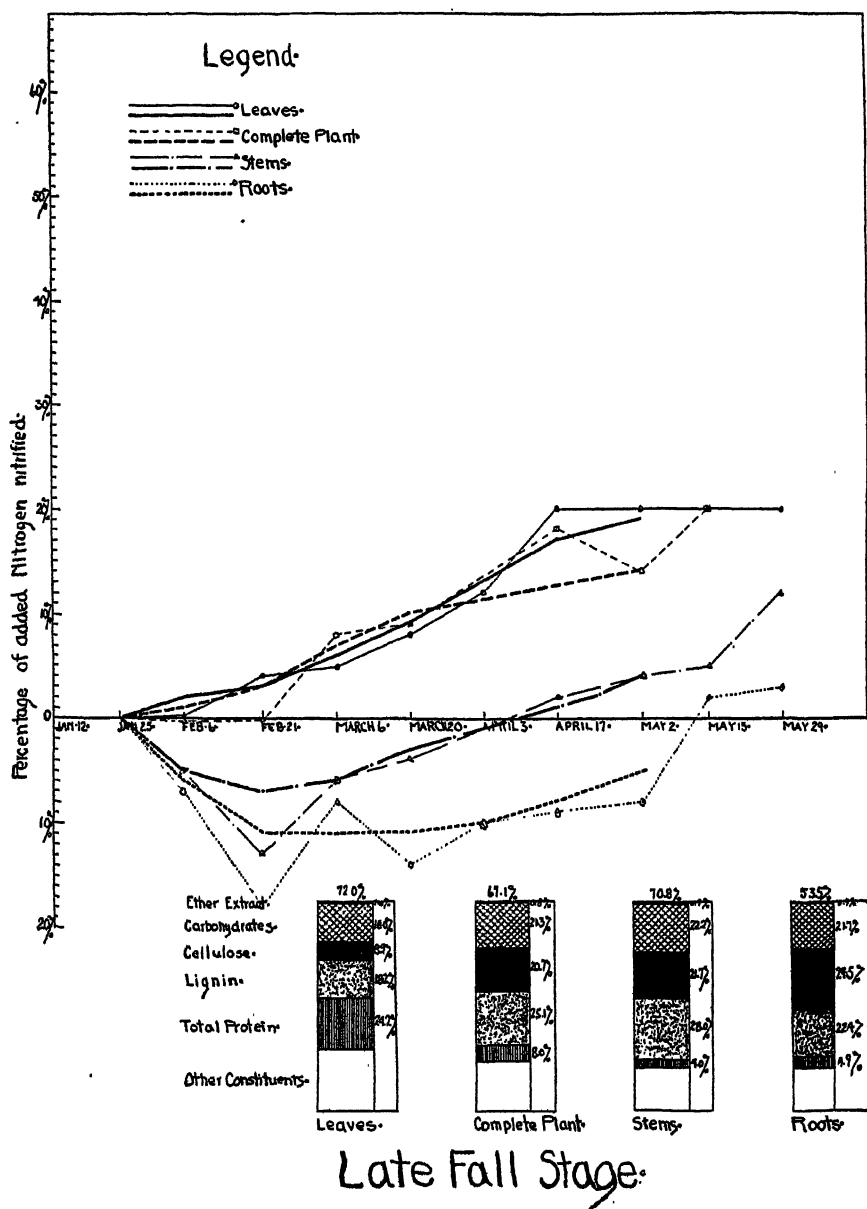


FIG. 4. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN A *late fall growth stage* OR AFTER FIRST FROST

maintain that 1.7 per cent nitrogen in the dry matter is just sufficient to cover the requirement of the microorganisms which are active in the decomposition of the plant (rye) material within a period of four weeks. Further, they contend that if the plant contains more than 1.7 per cent nitrogen, the excess nitrogen is rapidly liberated in an available form even within the first four weeks of decomposition. Thus, the complete *Crotalaria* plant and the *Crotalaria* leaves with a higher percentage of nitrogen than that mentioned in the foregoing, show a decidedly rapid accumulation of nitrate during the entire decomposition period, while the stems and roots with a percentage of nitrogen lower than 1.7 per cent, show an actual utilization of soil nitrogen during the early stages of decomposition.

This rate of nitrate accumulation in soil manured with the different plant parts is further substantiated by results by Joshi (4). This worker obtained

TABLE 5
Ratios of nitrogen to cellulose and easily hydrolyzable carbohydrates

| STAGE OF GROWTH | RATIO TOTAL NITROGEN TO CELLULOSE | | | | RATIO TOTAL NITROGEN TO EASILY HYDROLYZABLE CARBOHYDRATES | | | |
|------------------|-----------------------------------|----------------|--------|--------|---|----------------|--------|--------|
| | Leaves | Complete plant | Stems | Roots | Leaves | Complete plant | Stems | Roots |
| Succulent..... | 1:1.5 | 1: 4.7 | 1:14.5 | 1:16.4 | 1:4.9 | 1: 7.1 | 1:13.0 | 1:14.3 |
| Early bloom..... | 1:1.4 | 1: 5.6 | 1:14.5 | 1:17.0 | 1:3.6 | 1: 7.9 | 1:15.7 | 1:18.6 |
| Seed pod..... | 1:3.2 | 1: 7.8 | 1:19.8 | 1:25.4 | 1:5.7 | 1:10.7 | 1:24.7 | 1:28.1 |
| Late fall..... | 1:1.8 | 1:11.7 | 1:20.2 | 1:28.7 | 1:3.7 | 1:12.1 | 1:20.8 | 1:21.1 |
| Average..... | 1:2.0 | 1: 7.5 | 1:17.2 | 1:21.9 | 1:4.5 | 1: 9.5 | 1:18.6 | 1:20.5 |

a more rapid accumulation of nitrates in the soil from the incorporation of leaves of sann-hemp (*Crotalaria juncea*) than from the roots and stems of the same plant at one stage of growth. He also obtained similar results with dhaincha, guvar, and cowpeas. Joshi (4) indicated that the higher nitrogen content of the leaves of these plants was responsible for the more rapid accumulation of nitrates following their incorporation with the soil.

Bal (2), studying the decomposition of sann-hemp (*Crotalaria juncea*) at different stages of growth, found that the leaves decomposed more rapidly than did the stems of this plant. Studies of the nitrate accumulation from the complete plant as compared with that from the leaves and stems added to the soil separately showed that the leaves decomposed more rapidly than the complete plant, which decomposed more rapidly than the stems. In analyzing the data in the decomposition of the complete plant, Bal, calculating the relative percentage of nitrogen in the complete plant which was obtained from the stems and leaves and utilizing the results obtained on the incorporation of these plant parts separately, arrived at the conclusion that the stems had no influence upon the nitrate accumulation from the leaves when the stems and leaves were added together (as a complete plant) to the soil.

By a similar calculation, the roots, stems, and leaves of *Crotalaria striata* when incorporated with the soil separately and when added to the soil together (as a complete plant) produced nitrate accumulations analogous to those reported by Bal. Table 6 gives the percentages of the total nitrogen nitrified from the complete plant as observed experimentally together with the calculated percentages, the figures obtained upon the incorporation of the individual plant parts being utilized separately. Within the limits of experimental error, these calculated figures indicate that the roots and stems do not decrease the degree of nitrate accumulation from the leaves at the various growth stages of *Crotalaria striata*. Apparently the nitrogen (nitrates) of the original soil, which, as stated in the foregoing, is taken into consideration in these calculations, is sufficient to supply the needs of the organisms for the decomposition of the roots and stems.

The change in the degree of nitrate accumulation through the successive growth stages

The degree of nitrate accumulation in soils incorporated with the complete *Crotalaria* plant or any one of its parts appears to be associated with the ratio of nitrogen to carbohydrate materials, i.e., easily hydrolyzable carbohydrates and cellulose. Consequently, the change in the rate of nitrate accumulation in the soil incorporated with the complete plant or any one of its parts should vary at successive growth stages with the deviation in the composition of the plant or its parts through these growth stages.

A high rate of nitrification took place in the soil when it was incorporated with *Crotalaria* leaves in the succulent growth stage (fig. 5). A very similar rate of nitrification is obtained when the soil was manured with leaves of the *Crotalaria* plant in the early bloom stage or with those from the plant in the late seed pod stage. This increased degree of nitrate accumulation appears to be associated with a narrow ratio between the nitrogen and carbohydrate materials (easily hydrolyzable carbohydrates, cellulose) in the leaves. A similar ratio between the nitrogen and carbohydrates was found in the leaves of the plant in its late fall condition—after frost—but the degree of nitrification in the soil manured with these leaves was somewhat less than that of those from the plants in the first three growth stages. This difference is undoubtedly associated with the decreased percentage of plant moisture in the latter leaves.

This dependence of the degree of nitrate accumulation in the soil from plant material incorporated therein on the moisture content of such plant material is further substantiated by Hutchinson and Milligan (3) from whom we quote as follows:

The successful decomposition of the buried green crop has been shown to be determined largely by the presence of an adequate supply of water for the needs of the saprophytic organisms upon which this process depends. It is therefore to be expected that the moisture content of the green plant at the time of burial will materially affect the result, as will also the condition of the tissues in this respect. Thus, the decomposition of the younger plants is

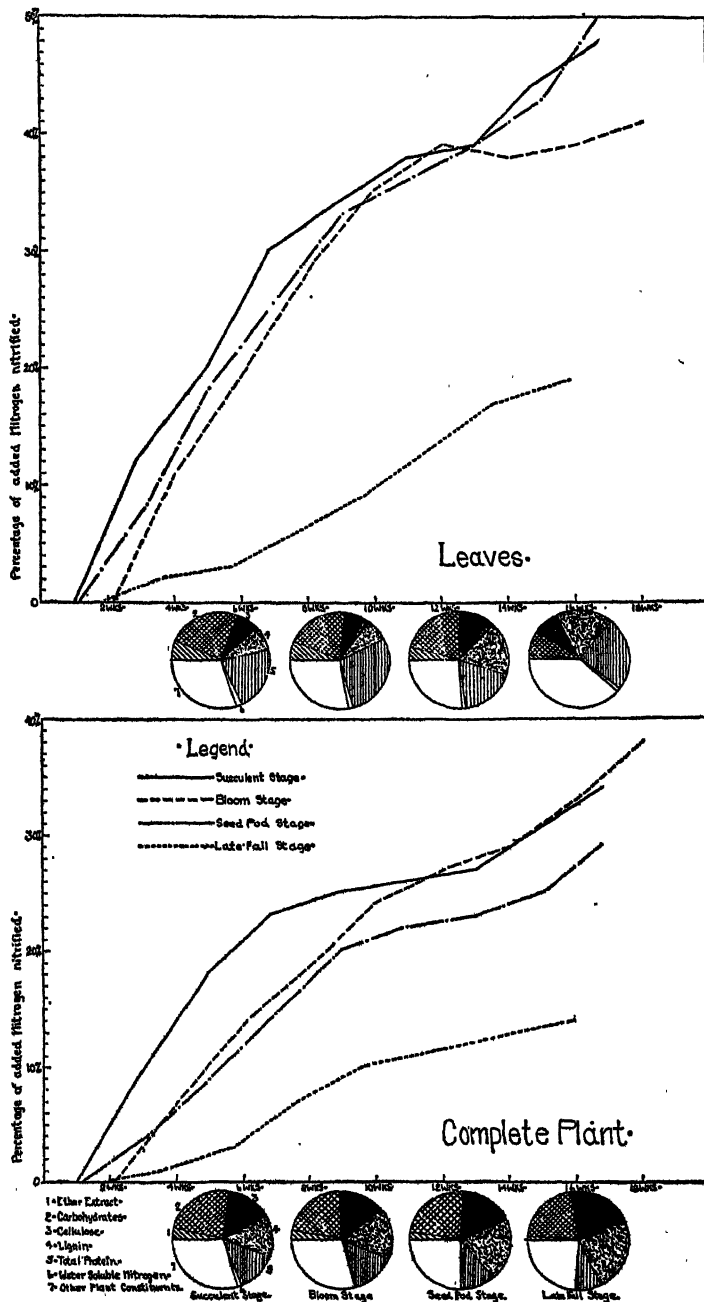


FIG. 5. PERCENTAGES OF NITROGEN NITRIFIED FROM THE LEAVES AND THE COMPLETE PLANT WHEN INCORPORATED WITH NORFOLK SANDY SOIL AT DIFFERENT GROWTH STAGES AS CORRELATED WITH THE COMPOSITION OF THE PLANT AT SUCH GROWTH STAGES

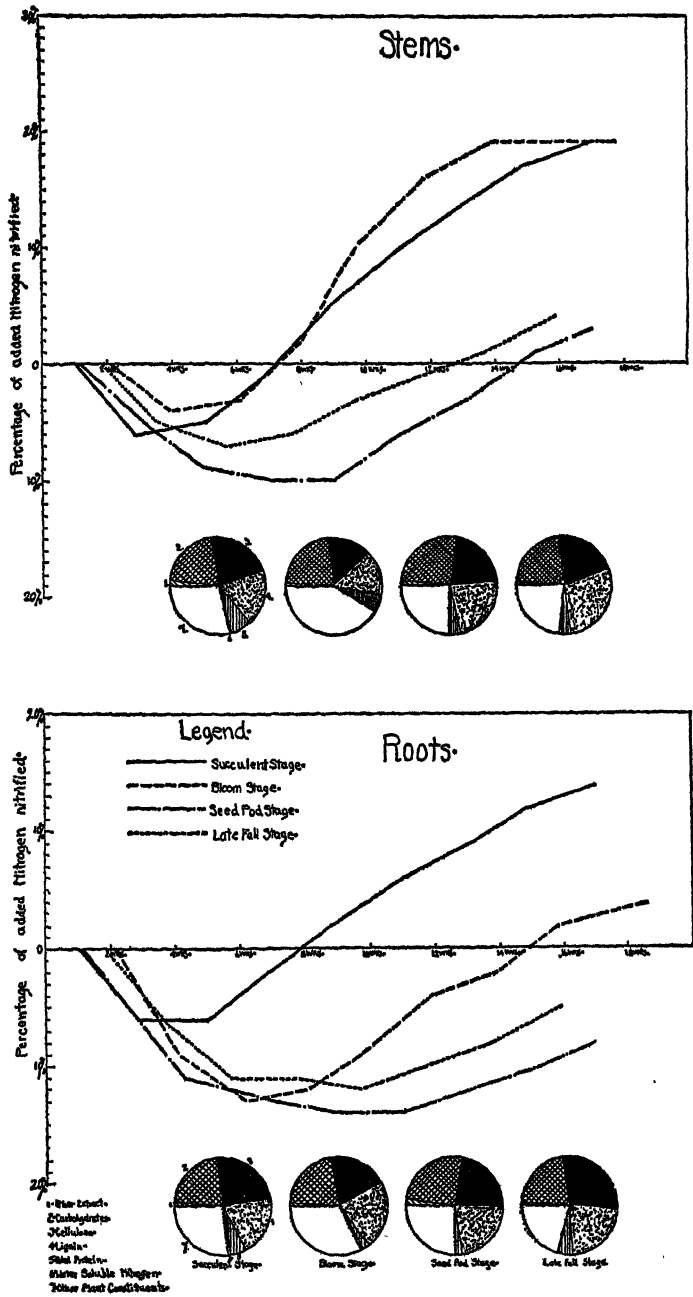


FIG. 6. PERCENTAGES OF NITROGEN NITRIFIED FROM THE STEMS AND ROOTS OF THE CROTA-LARIA PLANT WHEN INCORPORATED WITH NORFOLD SANDY SOIL AT DIFFERENT GROWTH STAGES AS CORRELATED WITH THE COMPOSITION OF THESE PARTS OF THE PLANT AT SUCH GROWTH STAGES

as much more rapid than that of the maturer ones as the water content of the former is higher than that of the latter, and those parts of the plant containing the highest proportion of cell sap have been found to decompose more rapidly than the maturer portions. This is well illustrated in the relative rates of nitrification of the plants in the three stages of growth under observation, and has a further bearing upon the effect of loss of moisture from the plants resulting from delay in burying after cutting down.

Whiting and Schoonover (10) observed that the decrease in the degree of nitrification from dehydration appears to be of a physical nature only.

The degree of nitrate accumulation in the soil manured with the complete plant at the successive stages of growth shows a progressive decrease with advancing maturity of the plant (fig. 6). This progressive decrease in the rate of nitrate accumulation is associated with a widening of the ratio between the nitrogen and the carbohydrate material (easily hydrolyzable carbohydrates, cellulose) from that in the succulent stage to that in the late fall stage, or for nitrogen to cellulose from 4.7 (succulent stage) to 5.6 (early bloom) to 7.8 (seed pod), to 11.7 (late fall), or for nitrogen to easily hydrolyzable carbohydrates from 7.1 (succulent stage) to 7.8 (early bloom stage) to 10.7 (seed pod) to 12.1 (late fall). These changes in composition are chiefly found in the stems and roots of the plant.

The time which elapses before nitrate accumulation takes place in the soil upon the incorporation of stems and roots of the plant of different growth stages is correlated with the width of the ratio between the total nitrogen and carbohydrates (see fig. 6 and Table 5). After the elapse of this period necessary for a positive accumulation of nitrates, there is a progressive increase in the degree of nitrate accumulation for the stems and roots of each of the different growth stages of the plant, the more rapid accumulation taking place with those stems and roots having a narrower ratio of nitrogen to carbohydrate material (succulent and early bloom stage).

Immediately after the incorporation of the stems and roots of the *Crotalaria* plant, a rapid and marked utilization of the nitrates of the soil is apparent. Subsequently, the amounts of nitrates utilized by the organisms in their decomposition of the plant materials gradually decrease. It appears that this early and large utilization of the nitrates of the soil is associated with the decomposition of the easily hydrolyzable carbohydrates of the stems and roots; subsequently to this the cellulose decomposing organisms utilize the nitrates until the ratio of nitrogen in the decomposing plant material to the microbial bodies is such that a positive accumulation of nitrates occurs.

Recovery of nitrogen

The accumulation of nitrates in the soil resulting from the incorporation of the *Crotalaria* plant or any one of its parts at the different growth stages is further illustrated by the response of the growths of Sudan grass in the soils thus treated. All the soil pots in which nitrate accumulation studies were made after the incorporation of the complete plant or any one of its parts,

were seeded with Sudan grass. Five plants were grown in each pot. Successive cuttings of this grass were made during the season at the maximum growth stage. The total yields from the pots are recorded in table 7. The percentage of nitrogen on a sample taken from the accumulated yields from each pot was determined. The percentage of nitrogen recovered in the Sudan grass from the different pots in which the soil had been manured with the complete plant or any one of its parts is likewise tabulated. From these data, it may be observed that the recovery of nitrogen in the Sudan grass is correlated with the degree of nitrate accumulation in the soil treated with the complete plant or any of its parts. This in turn is again correlated with the composition of the complete plant or any one of its parts at the different growth stages.

TABLE 6

Calculated and observed percentages of nitrogen nitrified when complete plant is incorporated with the soil

| TIME AFTER INCORPORATION | SUCCULENT STAGE | | BLOOM STAGE | | SEED POD STAGE | | LATE FALL STAGE | |
|-----------------------------|-----------------|----------|-------------|----------|----------------|----------|-----------------|----------|
| | Calculated* | Observed | Calculated* | Observed | Calculated* | Observed | Calculated* | Observed |
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| 2 | 7.7 | 9.4 | 6.9 | 7.3 | 4.5 | 4.0 | -1.4 | 0.9 |
| 4 | 13.9 | 17.5 | 13.7 | 13.6 | 10.8 | 9.3 | -2.2 | 2.7 |
| 6 | 22.2 | 22.9 | 21.5 | 19.1 | 16.4 | 15.1 | -0.3 | 6.7 |
| 8 | 26.7 | 25.1 | 27.7 | 23.7 | 21.6 | 19.9 | 2.4 | 10.4 |
| 10 | 30.5 | 25.9 | 32.2 | 26.9 | 23.2 | 21.8 | | |
| 12 | 32.4 | 25.9 | 32.2 | 29.3 | 27.9 | 23.4 | | |
| 14 | 35.9 | 29.6 | | | 31.8 | 24.5 | 8.4 | |
| 16 | 40.7 | 33.8 | 34.6 | 37.7 | 37.3 | 28.6 | 10.9 | 14.43 |

* Calculated from the percentages of nitrogen nitrified when the leaves, stems, and roots were incorporated separately and from the percentages of nitrogen in the complete plant in the form of these plant parts.

APPLICATION

The utilization of the *Crotalaria* plant for the sandy soils with a low organic matter content is becoming a general farm and grove practice. The introduction of this summer leguminous plant has created a number of problems relative to its proper use in the numerous farm and grove practices. Since the accumulation of nitrogen in the soil subsequent to the incorporation of a green manure crop must coincide with the maximum demand for nitrogen of the crops to be benefited, it follows that the planting and turning under of a green manure crop must be adjusted to the various cultural practices of such crops.

All other conditions being favorable, if a rapid accumulation of nitrates is desired, as in the case of vegetable crops, then the *Crotalaria* plant should be incorporated with the soil in its early growth stages. If, on the other hand, a retarded accumulation of nitrate is desired, so that the crop or tree may make use of the nitrogen in the spring months, the plant should be in an advanced

stage of growth and dry before it is incorporated with the soil. The present study of the composition relation of the *Crotalaria* plant to the accumulation of nitrates in the soil may be of value in the adjustment of this leguminous plant in the many varied cultural practices in which it will no doubt be used.

TABLE 7

The yield of dry matter, percentages of nitrogen in the dry matter, and percentages of added nitrogen recovered in Sudan grass planted on soils receiving green manures in the form of Crotalaria leaves, complete plants, stems, and roots at different growth stages

| PLANT OR PLANT PARTS INCORPORATED | AVERAGE YIELD OF SUDAN GRASS | AVERAGE NITROGEN IN GRASS | NITROGEN RECOV- ERED | INCREASED NITROGEN RECOV- ERED | NITROGEN ADDED AS GREEN MANURE | NITROGEN RECOV- ERED |
|-----------------------------------|---------------------------------------|---------------------------------|----------------------------|---|---|----------------------------|
| | gm. | per cent | gm. | gm. | gm. | per cent |
| <i>Succulent stage</i> | | | | | | |
| Nothing..... | 6.5 | 0.605 | 0.039 | | | |
| Leaves..... | 34.9 | 0.990 | 0.346 | 0.307 | 0.827 | 37.1 |
| Complete plant..... | 34.1 | 0.833 | 0.284 | 0.245 | 0.531 | 46.1 |
| Stems..... | 19.9 | 0.535 | 0.106 | 0.067 | 0.250 | 26.8 |
| Roots..... | 19.9 | 0.508 | 0.101 | 0.062 | 0.279 | 22.2 |
| <i>Bloom stage</i> | | | | | | |
| Nothing..... | 6.5 | 0.598 | 0.039 | | | |
| Leaves..... | 35.3 | 1.258 | 0.444 | 0.405 | 1.108 | 36.6 |
| Complete plant..... | 33.8 | 0.795 | 0.269 | 0.230 | 0.665 | 34.6 |
| Stems..... | 23.2 | 0.620 | 0.144 | 0.105 | 0.321 | 32.7 |
| Roots..... | 16.8 | 0.609 | 0.102 | 0.063 | 0.267 | 23.6 |
| <i>Seed pod stage</i> | | | | | | |
| Nothing..... | 6.5 | 0.605 | 0.039 | | | |
| Leaves..... | 44.9 | 1.038 | 0.466 | 0.427 | 1.205 | 35.4 |
| Complete plant..... | 32.3 | 0.873 | 0.282 | 0.243 | 0.793 | 30.6 |
| Stems..... | 17.9 | 0.583 | 0.104 | 0.065 | 0.411 | 15.8 |
| Roots..... | 14.1 | 0.533 | 0.075 | 0.036 | 0.336 | 10.7 |
| <i>Late fall stage</i> | | | | | | |
| Nothing..... | 14.7 | 0.604 | 0.089 | | | |
| Leaves..... | 56.8 | 1.150 | 0.653 | 0.564 | 3.470 | 16.3 |
| Complete plant..... | 34.9 | 0.960 | 0.335 | 0.246 | 1.194 | 20.6 |
| Stems..... | 27.9 | 0.823 | 0.230 | 0.141 | 0.758 | 18.6 |
| Roots..... | 14.3 | 0.559 | 0.080 | | 0.546 | 0.0 |

All results averages of duplicate treatments.

SUMMARY

A study has been made of the composition of the *Crotalaria* leaves, stems, roots, and complete plant at different stages of growth with special reference to nitrogen and carbohydrate compounds. The plants were analyzed in the

succulent, bloom, seed pod, and late fall growth stages. A study of the course of the accumulation of nitrates in Norfolk sandy soil upon the incorporation of the complete plant and each of its parts was made.

In any growth stage, there is a progressive decrease in percentage of nitrogen in the complete plant and its separate parts in the following order: leaves, complete plant, stems, and roots. There is a progressive increase in the percentage of cellulose and lignin in the order: leaves, complete plant, stems, roots. The carbohydrate percentage in the plant and its parts was rather constant. This resulted in a narrow ratio between total nitrogen and carbohydrates and cellulose for the leaves, which gradually widened in the complete plant, stems, and roots, respectively.

The accumulation of nitrates following the incorporation of the complete plant or any of its parts was most rapid from the leaves, progressively decreasing from the complete plant, stems, and roots in the order named, except with the leaves in the late fall stage, which varied little from that of the complete plant. The stems and roots show a utilization of the nitrate nitrogen of the soil in the early stages of decomposition. There is relatively little variation in the percentages of nitrogen and carbohydrates in the leaves of the plant through the successive growth stages but there is some increase in the percentages of lignin and cellulose as the plant approaches maturity. There is little variation in the rapidity of the nitrification of the nitrogen added as leaves, with the exception of the leaves in the late fall growth stage, where desiccation occurred. With the complete plant through the successive growth stages, there is a progressive increase in percentage of lignin and cellulose with a corresponding decrease in percentage of nitrogen, whereas the percentage of the carbohydrates remains rather constant.

There is a progressive decrease in the rapidity of the nitrification of the nitrogen added to the soil from the complete plant through the successive stages of growth, i.e., succulent, bloom, seed pod, and late fall, in decreasing order.

In the first two growth stages (succulent and bloom) there is little variation in the percentage of nitrogen, cellulose, and lignin in the stems and roots, whereas during the two latter growth stages there is an increase in percentage of cellulose and lignin and a decrease in the percentage of nitrogen. The carbohydrates were rather constant in percentage for all growth stages.

There is only a slight variation in the course of nitrification of nitrogen added to the soil as stems in the succulent and bloom stages. This course showed a more rapid accumulation of nitrates from these stems than from the stems of the seed pod and late fall stages. The time required for a positive accumulation of nitrates after the incorporation of the stems was shorter for the succulent and bloom stage stems than for the seed pod and late fall stems. Similar results were obtained with the roots.

Possible practical applications for the foregoing results are given.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: II. CATAPHORESIS, FLOCCULATION, AND DISPERSION¹

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In the study of colloidal behavior the problem of stability is of fundamental importance. It is generally assumed that the stability of a suspension is directly connected with the potential difference within the double layer. This represents the cataphoretic potential ζ which may be calculated from the cataphoresis of the particles according to the Helmholtz-Perrin formula:

$$\zeta = \frac{4 \pi \eta v}{H D}$$

where v = velocity of the particles, η = viscosity of liquid, H = potential gradient in volt/cm., and D = dielectric constant of liquid.

With $\eta = 0.01$ and $D = 81$ for water at room temperature, the potential expressed in millivolts will be found by multiplying the velocity of the particles, expressed in μ per second, by the factor 14. It has recently been brought out however, by Debye and Hückel (6) that for spherical particles the factor 4 in the above equation should be 6. This would make the cataphoretic potentials reported in earlier papers 50 per cent larger. Multiplying the velocity by 21 instead of by 14 would therefore include this correction. It is in all cases best to report the observed quantities, i.e., the velocities.

In connection with the foregoing work it was deemed of utmost importance to determine the influence of the different electrolytes on the stability of the suspensions and on the cataphoretic migration. This was done as reported in previous publications (21) a cataphoresis cell of the same construction being employed.

THE EFFECT OF BASES AND SALTS

Table 17 shows the cataphoresis of bentonite in various concentrations of the chloride, sulfate, and ferrocyanide of sodium. The concentrations at which the suspensions flocculated are indicated by x 's, one x signifying a beginning of flocculation while four x 's stand for complete flocculation. The suspensions were prepared by mixing 25 cc. of a 0.2 per cent stock suspension with 25 cc. of the solutions. After standing over night the flocculation was observed, the

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tubes were shaken, and the cataphoresis measurements made. The latter represent an average of three measurements in each direction, the current being reversed each time.

Let us first consider the flocculation. In a concentration of N 0.02, flocculation was complete in the chloride, about half complete in the sulfate, and had not begun in the ferrocyanide solution. In N 0.03 it was complete in the sulfate and about half complete in the ferrocyanide solution. In the ferrocyanide solution flocculation was complete in the N 0.04 concentration. There is therefore an unmistakable valence effect and the most significant fact is that this effect agrees, one could almost say quantitatively, with the suppression of the Donnan potential, the swelling, and the viscosity, as shown in part I of this paper. Flocculation being in effect a suppression of the stability, one is led to

TABLE 17
Flocculation and cataphoresis of bentonite

| CONCENTRATION | NaCl | | | Na ₂ SO ₄ | | | Na ₂ Fe(CN) ₆ | | |
|---------------|--------------|---------------------------|--------------------------|---------------------------------|---------------------------|--------------------------|-------------------------------------|---------------------------|--------------------------|
| | Flocculation | μ /sec. 1 volt/cm. | Millivolts calculated | Flocculation | μ /sec. 1 volt/cm. | Millivolts calculated | Flocculation | μ /sec. 1 volt/cm. | Millivolts calculated |
| N | | | | | | | | | |
| 0.0 | 0 | -2.77 | -58.2 | | | | | | |
| 0.001 | 0 | -2.80 | -58.8 | 0 | -3.10 | -65.0 | 0 | -3.36 | -70.5 |
| 0.004 | 0 | -2.85 | -59.8 | 0 | -3.16 | -66.4 | 0 | -3.52 | -74.0 |
| 0.01 | 0 | -2.85 | -59.8 | 0 | -3.33 | -70.0 | 0 | -3.52 | -74.0 |
| 0.02 | xxxx | -3.16 | -66.4 | xx | -3.61 | -75.7 | 0 | -3.79 | -79.5 |
| 0.03 | xxxx | -3.53 | -74.0 | xxxx | -3.87 | -81.3 | xx | -3.93 | -82.5 |
| 0.04 | xxxx | -3.73 | -78.3 | xxxx | -3.96 | -83.1 | xxxx | -4.03 | -84.5 |

the conclusion that a suppression of one is accompanied by a suppression of the other of these phenomena and that they must all be closely related to one another.

A quantitative relationship of the valence of the ions with the same sign of charge as the colloid has never been established, for, although it is generally admitted that the nature of the ions with the same sign of charge does influence the stability, attention has chiefly been focused on the ions of opposite sign, the valence effect of which are usually much more pronounced. As far as the Donnan equilibrium has anything to do with the action of electrolytes on the stability of colloids, nothing but the valence of the ions is of importance. In the case of an electronegative colloid the equivalent concentration of an electrolyte with a divalent anion would have to be about 50 per cent greater than the concentration of an electrolyte with a monovalent anion (the cation being the same) in order to produce the same effect. This is approximately the relationship between the flocculating concentrations of the chloride and the sulfate solutions in the preceding experiment. In the case of a solution with a divalent cation only approximately one-half the equivalent concentration of a

solution with a monovalent cation (the anion being the same) should be necessary to produce the same effect as far as the influence of the Donnan equilibrium is concerned. The exact relationship will depend on the ratio $\frac{z}{y}$.

The first relationship, that of the anions, can only hold when the anions do not enter into combination with the colloid, as in the preceding case. It is only the free, uncombined fraction of the electrolyte which takes part in the Donnan distribution. The second relationship never holds. The flocculating power of a divalent cation is usually ten to twenty times as great as that of a monovalent cation. (We continue our reference to a negative colloid.) The reason is that the cation, the ion of opposite sign of charge, always combines with the colloid by exchange. Each new combination yields a new complex, a different micelle and a different colloid having different properties. In these combinations any property of the ions may be of importance, such as size, electro-affinity, activity, hydration, and valence. It is in this relationship that the Hofmeister or lyotropic ion series become manifest. We can no more deny the influence of these series than we can deny the influence of the Donnan equilibrium—both are present but very often the effect of one will be found to obscure the effect of the other.

Comparing the cataphoretic potential in the three solutions at the concentrations sufficient to flocculate we do not find a constant critical potential as is generally assumed on the basis of the much quoted experiment of Powis (26) on an oil emulsion. The critical cataphoric potential is lowest in the chloride solution and highest in the ferrocyanide. As far back as 1920 while working in the laboratories of Professor P. Ehrenberg in Göttingen the author (15) established this anomaly for the first time. These observations have recently been verified by other investigators (12, 28). Numerous experiments, some of which will be here presented, show that, as far as soil colloids are concerned, there is no one critical cataphoretic potential but that this potential is different for each pair of ions.

Following the work of Hardy (10) it was believed and has, until recently, frequently been stated that flocculation takes place only at the isoelectric point. But the work of Burton (3), Powis, and others later showed that the flocculating particles retained a considerable part of their charge. The view that the stability is directly related to the cataphoretic potential is accepted by most authorities although it is not clear how the P. D. between two concentric layers can give rise to an external repulsion. Porter and Hedges explain how the mutual action between the particles may be of the nature of an attraction instead of a repulsion. They state (25):

When the existence of this double layer is recognized, the electrical forces between the particles become zero, except in so far as relative displacement takes place by induction between two members of a layer so as to give it an electrical moment. In this case the force between two such doublets in the equilibrium state will, on the average, be an attraction and not a repulsion.

In order to connect the charge of the double layer directly with the stability, it is argued that the spheres of like signs are unable to penetrate one another and thus prevent actual collision of the particles. But is flocculation the result of such actual collisions? Consider the large volume occupied by the flocculated material, which may amount to over 100 cc. per gram solids (1). Or consider the setting into a gel of a 5.36 per cent suspension of bentonite when sufficient NaOH was added to cause flocculation (*compare* table 16, part I). Dividing by 2.65, the approximate sp. gr., we find that the solid particles occupy only a little over 2 per cent of the gel. The particles must, therefore, be comparatively far apart from one another, yet they are linked together forming a continuous, more or less rigid network. This shows definitely that it must be the bulky micelles and not their nuclei, i.e., the particles themselves, which adhere in the process of flocculation. In other words, linkage is established between the outer spheres of the micelles, that is, in the very regions in which the repelling force is supposed to reside.

Another irregularity from the point of view of the generally accepted theory on stability appears in table 17, in that the cataphoretic P.D., as calculated from the speed of migration, is higher in the case of the flocculated material than in the absence of electrolytes. This phenomenon was first observed by Anderson and Mattson when studying the effect of methylene blue on the same sample of bentonite (1). We shall meet with similar cases in connection with soil colloids later.

An increase in the charge in low concentrations of the salts of the alkali metals has quite generally been met with. The logical interpretation of this behavior has been the assumption that the anion is more extensively adsorbed in low concentrations, whereas in higher concentrations the cation adsorption gradually catches up with the adsorption of the anion. This would nicely explain the rise and fall in the migration velocities but it is not in harmony with the observations here made that neither the Cl , NO_3 , SO_4 or $\text{Fe}(\text{CN})_6$ ions are adsorbed by the colloid. On the contrary, we have seen that these ions are negatively adsorbed because of the Donnan distribution. They therefore can not cause an increase in the cataphoretic potential by a preferential adsorption. Neither is the assumption that the cation adsorption gains on the anion adsorption in higher concentrations, in harmony with the foregoing results, which show that the micellar cation concentration, i.e. the value of z , increases with the concentration of the added electrolyte. This release of cations must give rise to a greater number of free charges on the surface of the particle and should, according to formula (C), increase the cataphoretic potential, provided the thickness of the double layer is not at the same time decreased.

The question arises: Is the suppression of the stability caused by the free uncombined ions or it is caused by an adsorption of ions of opposite charge? If the suppression is related to the Donnan equilibrium, as is the suppression of the swelling viscosity and the Donnan potential, then it is the free electrolyte which is the deciding factor. In as far as the suppression of the stability

depends on a neutralization of the free charges on the surface of the particles, we must look for an explanation in the adsorption, i.e. in the association tendency of the ions of opposite sign of charge. We know that the P.D. between the micellar solution and the outside liquid, i.e. the Donnan potential, must be proportional to the micellar ion concentration, i.e., to the number of cations dissociated by the particles. The divalent cations are less dissociated than the monovalent cations and accordingly give rise to a lower P.D. This is in harmony with the dispersibility of soil materials saturated with different cations. In the case of Na-, K-, Mg-, Ca-, and Ba-saturated soil samples, Gedroiz (8) found the following order in the amount of material dispersed $\text{Na} > \text{K} > \text{Mg} > \text{Ca} > \text{Ba}$. As to the *suppression* of the Donnan P.D. by the addition of electrolytes, we know that, apart from any change in the nature of the micelle by an exchange of cations, the free electrolyte suppresses the P.D. according to the valence of the ions and in proportion to the concentration, as has been shown.

By taking into account the different tendencies of the various cations to remain dissociated in the micellar solution and by recognizing the valence effect on the Donnan distribution of the free electrolyte, we can easily formulate a theory which will as satisfactorily explain the stability of a suspension as we are able to explain the swelling and the viscosity. In the above experiment the cation (Na) of the added electrolytes was the same as that in the colloid. The cation influence was therefore the same in all cases. The influence of the valence of the anions, none of which were adsorbed, was almost quantitatively what it should be if the Donnan equilibrium were the sole factor. But so far nothing explains the anomalies of the cataphoretic potential. Before proceeding with the discussion it will therefore be profitable to give some additional data on flocculation and cataphoresis.

Table 18 shows the effects of KCl on the untreated, the electrodialyzed, the Na-saturated, and the Ca-saturated Sharkey clay soil colloid.

The electrodialyzed colloid flocculated in a concentration of KCl about four times weaker than the concentrations in which the base-saturated sample flocculated. This greater sensitiveness of the unsaturated (H-saturated) sample is probably due to the exchange acidity, which mobilizes the trivalent cations Al and Fe (22). This emphasizes the importance of the condition of the colloid. Comparable results can only be obtained by working with material saturated with the same cation as that of the electrolyte employed. There is here no apparent difference between the stability of the Na- and Ca-saturated suspensions, but that is chiefly because the suspensions were so dilute that the small quantity of Ca ions displaced by the K ions did not materially affect the flocculation. In more concentrated suspensions the effect of cation exchange will be pronounced.

The influence of a displacement of Ca ions by K ions shows itself however in the P.D., which increases from 40.1 to a maximum of 50.6 millivolts in the case of the Ca-saturated colloid. In all other cases the P.D. decreases with an

TABLE 18
Sharkey soil colloid—KCl System
 (0.4 gm. colloid per liter)

| MILLIEQUIVA- LENTS IN LITER | FLOCCULATION | μ /SEC. 1 VOLT/CM. | P.D. MILLI- VOLTS | FLOCCULATION | μ /SEC. 1 VOLT/CM. | P.D. MILLI- VOLTS |
|--------------------------------|------------------|---------------------------|----------------------|-------------------------|---------------------------|----------------------|
| | Original colloid | | | Electrodialyzed colloid | | |
| 0.0 | 0 | -2.70 | -56.7 | 0 | -3.20 | -67.2 |
| 0.5 | 0 | -2.85 | -58.8 | 0 | -3.10 | -65.1 |
| 1.0 | 0 | -2.85 | -58.8 | x | -2.60 | -54.6 |
| 4.0 | 0 | -2.70 | -56.7 | xxxx | -2.40 | -50.5 |
| 8.0 | 0 | -2.58 | -54.2 | xxxx | -2.06 | -43.3 |
| 12.0 | xx | -2.40 | -50.5 | xxxx | -1.94 | -40.7 |
| 16.0 | xxx | -2.35 | -49.3 | xxxx | -1.81 | -38.0 |
| 20.0 | xxxx | -2.35 | -49.3 | xxxx | -1.76 | -37.0 |
| | Na-saturated | | | Ca-saturated | | |
| 0.0 | 0 | -3.65 | -76.6 | 0 | -1.91 | -40.1 |
| 0.5 | 0 | -3.48 | -73.1 | 0 | -1.93 | -40.5 |
| 1.0 | 0 | -3.18 | -66.7 | 0 | -2.11 | -44.3 |
| 4.0 | 0 | -3.03 | -63.7 | 0 | -2.32 | -48.7 |
| 8.0 | 0 | -2.91 | -61.2 | 0 | -2.41 | -50.6 |
| 12.0 | xx | -2.61 | -54.8 | xx | -2.36 | -49.5 |
| 16.0 | xxx | -2.52 | -53.0 | xxx | -2.30 | -48.3 |
| 20.0 | xxxx | -2.58 | -54.2 | xxxx | -2.26 | -47.5 |

TABLE 19
Influence of CaCl_2 and of $\text{Ca}(\text{OH})_2$ on the Sharkey colloid
 (0.4 gm. colloid per liter)

| CaCl_2 MILLIEQUIVA- LENTS IN LITER | Ca-SATURATED | | | ELECTRODIALYZED | | |
|---|--------------|---------------------------|--------------------|-----------------|---------------------------|--------------------|
| | Flocculation | μ /sec. 1 volt/cm. | P.D. millivolts | Flocculation | μ /sec. 1 volt/cm. | P.D. millivolts |
| 0.0 | 0 | -1.91 | -40.0 | 0 | -3.20 | -67.2 |
| 0.2 | | | | 0 | -1.40 | -29.4 |
| 0.4 | 0 | -1.22 | -25.6 | xx | -1.05 | -22.0 |
| 0.5 | | | | xxx | -1.02 | -21.4 |
| 0.7 | | | | xxxx | -0.89 | -18.7 |
| 0.8 | x | -1.06 | -22.2 | | | |
| 1.2 | xxx | -1.03 | -21.6 | | | |
| 1.6 | xxxx | -0.97 | -20.4 | | | |
| $\text{Ca}(\text{OH})_2$ | | | | | | |
| 0.0 | 0 | -1.91 | -40.0 | 0 | -3.20 | -67.2 |
| 0.4 | 0 | -1.52 | -31.9 | 0 | -1.69 | -35.5 |
| 0.6 | x | -1.21 | -25.4 | | | |
| 0.8 | xxx | -1.09 | -22.9 | 0 | -1.44 | -30.2 |
| 1.0 | xxxx | -1.02 | -21.4 | x | -1.13 | -23.7 |
| 1.2 | xxxx | -0.93 | -19.5 | xxx | -0.98 | -20.6 |
| 1.4 | | | | xxxx | -0.95 | -19.9 |

increase in concentration. It appears that the cataphoretic potential depends on the nature of the adsorbed cation, whereas the *suppression* of the P.D. and the stability are governed by the free electrolyte. All the suspensions in this experiment flocculate at about 50 millivolts, but in the case of the Ca-saturated colloid, as in the above case of bentonite, flocculation takes place at a considerably higher P.D. than that of the original suspension containing no chloride. Again we find no parallelism between the cataphoretic potential and the stability.

TABLE 20
Influence of $AlCl_3$ and of methylene blue on the Sharkey soil colloid
(0.2 gm. colloid per liter)

| $AlCl_3$ MILLIEQUIVA- LENTS PER LITER | UNTREATED COLLOID | | | ELECTRODIALYZED COLLOID | | |
|---|-------------------|---------------------------|--------------------|-------------------------|---------------------------|--------------------|
| | Flocculation | μ /sec. 1 volt/cm. | P.D. millivolts | Flocculation | μ /sec. 1 volt/cm. | P.D. millivolts |
| 0.0 | 0 | -2.40 | -50.5 | 0 | -3.20 | -67.2 |
| 0.1 | x | -1.67 | -35.0 | xxx | -1.60 | -33.6 |
| 0.2 | xxxx | -1.62 | -34.0 | xxxx | -0.80 | -16.8 |
| 0.4 | xxxx | -0.47 | -9.9 | xxxx | -0.59 | -12.4 |
| 0.7 | xxxx | -0.18 | -3.8 | xxxx | -0.45 | -9.4 |
| 1.0 | xxxx | -slight | | xxxx | -0.17 | -3.6 |
| 1.5 | xxxx | +0.48 | +10.1 | xxxx | ± 0.0 | ± 0.0 |
| 2.0 | xxxx | +0.61 | +12.3 | xxxx | +0.14 | +2.9 |
| 4.0 | xxxx | +0.78 | +16.4 | xxxx | +0.49 | +10.3 |
| Methylene blue | | | | | | |
| 0.032 | 0 | -2.0 | -42.0 | x | -2.2 | -46.2 |
| 0.043 | 0 | -1.8 | -37.8 | xx | -2.0 | -42.0 |
| 0.054 | xx | -2.0 | -42.0 | xxx | -1.8 | -37.8 |
| 0.064 | xxx | -1.6 | -33.6 | xxxx | -1.1 | -23.1 |
| 0.080 | xxx | -1.4 | -29.0 | xxxx | -slight | |
| 0.107 | xxxx | -1.3 | -27.3 | 0 | +1.4 | +29.0 |
| 0.121 | xxxx | -0.8 | -16.8 | | | |
| 0.134 | xxxx | -slight | | | | |
| 0.139 | xxxx | +0.6 | +12.6 | | | |
| 0.145 | xxx | +1.1 | +23.1 | | | |
| 0.161 | 0 | +2.2 | +46.2 | (1) | +3.0 | +63.0 |

Table 19 shows the effect of $CaCl_2$ and $Ca(OH)_2$ on the Ca-saturated and electrodialysed Sharkey soil colloid.

As in the case of KCl , the unsaturated colloid is flocculated in a considerable lower concentration of $CaCl_2$ than is the Ca-saturated colloid, which, as already stated, must be because the exchange acidity liberates Al and Fe ions, resulting in the formation of electropositive oxychlorides. The weaker flocculating power of the chloride in the Ca-saturated colloid is not due to a peptizing effect of $Ca(OH)_2$, which in the saturated colloid is seen to flocculate even more powerfully than the chloride, but to the absence of an exchange acidity, and

its secondary products. In the case of the hydroxide the relationship is reversed the unsaturated material requiring about 0.4 milliequivalent base per liter more than the colloid already saturated with Ca. Since one liter contained 0.4 gm. colloid the difference is roughly equal to the quantity of base required to saturate the colloid, which is about 0.8 milliequivalents per gram. Experiments with more concentrated suspensions showed that the quantities of $\text{Ca}(\text{OH})_2$ required for flocculation increased progressively with the concentrations of the electrodialyzed material while the concentrations of the colloid already saturated with the base had very little influence on the concentration of CaCl_2 and $\text{Ca}(\text{OH})_2$ required for flocculation.

It may be stated as a general rule that the colloid concentration influences greatly the flocculating value of any electrolyte which is adsorbed by the colloid while this is not the case when the electrolytes are not adsorbed. This again supports the view that it is the free electrolyte which is responsible for the suppression of the P. D. and stability as demanded by the Donnan equilibrium. The nature of the adsorbed cation determines the initial charge; it is only the suppression of this charge which is governed by the free electrolyte.

Comparing the critical potential in the last two tables we find that although KCl flocculated at about 50 millivolts the chloride and hydroxide of calcium do not flocculate until the P.D. is reduced to 20 millivolts.

Table 20 shows the influence of AlCl_3 and of methylene blue (tetramethylthionin chloride, $\text{C}_{16}\text{H}_{18}\text{N}_3\text{S}\text{Cl} + 3\text{H}_2\text{O}$, M. W. = 373.6) on the Sharkey colloid. In the case of the action of AlCl_3 , there is a slight difference in the flocculating values and a considerable difference between the isoelectric points of the untreated or saturated colloid and the electrodialyzed or unsaturated colloid. On the basis of the author's previous work (21) this is easily explained. It has been shown that the products of hydrolysis of aluminum salts, i.e., the oxychlorides which constitute an electropositive sol, are far more active in suppressing the charge and stability of electronegative colloids than are the nonhydrolyzed salts themselves. In 0.2 gm. of the untreated neutral colloid there is $0.2 \times 0.8 = 0.16$ milliequivalent displaceable bases. In the first two concentrations of AlCl_3 , or up to 0.2 milliequivalent, the chloride is rendered inactive by the formation of the normal hydroxide, which is not electropositive. The effect is here merely due to the chlorides of the displaced base (displaced by H and not by Al). The effect of the AlCl_3 , which is indirect in the saturated colloid, is therefore here weaker than in the case of the unsaturated colloid as far as the lowest concentrations are concerned. In higher concentrations the AlCl_3 is only partly neutralized by the bases in the saturated colloid, resulting in the formation of the highly electropositive oxychloride. It requires therefore less AlCl_3 to neutralize the negative charge of the saturated than that of the unsaturated colloid. In the latter suspension the aluminum salt is much less hydrolyzed.

An instructive experiment with the unsaturated colloid- AlCl_3 system may be performed. If AlCl_3 , insufficient electrically to neutralize the negative

colloid, is added and if the chloride is then partly alkalized by the addition of NaOH, the floc becomes at once isoelectric or electropositive. We are confronted therefore with the paradox of seeing NaOH render a negative colloid isoelectric or even electropositive but if an excess of NaOH is added this base will act true to form and charge the colloid strongly electronegative and disperse it. The explanation is that in the partial alkalization we are chemically producing an electropositive sol whereas with an excess of alkali we destroy the electropositive behavior by forming the normal hydroxide of aluminum, which, like all other materials, is electronegative in NaOH. These facts are of the greatest importance for the process of water purification and in the reclamation of alkali soils in which these agents are employed.

The action of methylene blue on soil colloid has been discussed in two previous papers (16, 21). This cation is completely adsorbed over the entire range on the electronegative side of the isoelectric point. The quantity of the dye required to render the colloid isoelectric is therefore directly proportional to the quantity of colloid. In the case of neutral soil colloids, and in the absence of other electrolytes, this quantity of the dye was found to be equivalent to the quantities of exchangeable bases, so that, in the case of different colloids, the quantities of methylene blue required to neutralize their negative charge is a direct measure of their exchange capacity. The methylene blue cation, which actively displaces the common cations, does not seem to be dissociated by the particles. When all the common cations have been displaced the colloid therefore will be isoelectric. This explains the equivalence. The adsorption proceeds beyond the isoelectric point but becomes more and more incomplete with an increase in the positive charge. In the electropositive condition the anion, i.e., the Cl ion, must make up the micellar ion atmosphere.

It will be seen that the unsaturated colloid requires less methylene blue both for flocculation and for electrical neutralization. This is, as already explained, due to the exchange acidity, which here attains a maximum because of the great activity of this cation. Furthermore the exchange capacity of the colloid has been shown to decrease with the pH (19). The addition of electrolytes to the methylene blue-soil colloid system has the following effect: Bases and salts with di- and poly-valent anions cause a greater quantity of the dye to be required for the electrical neutralization of the colloid. Acids and salts with di- and tri-valent cations have the opposite effect.

The critical P.D. in the preceding experiment we find to be about 30 millivolts.

Table 21 shows the action of the NaOH on the electrodialyzed and Ca-saturated Sharkey colloid. As is well known, the NaOH flocculates only in comparatively high concentrations but the Ca-saturated colloid is more sensitive than the electrodialyzed, or rather the Na-saturated, colloid. (The unsaturated colloid is of course immediately saturated with the added base.) The anomaly of the critical potential is here very great. The Na-saturated colloid flocculates

at as high a P.D. as 64 millivolts and the Ca-saturated at 56, which is again higher than the P.D. of the original stable suspension.

The preceding experiments show the influence of individual electrolytes on the stability and cataphoresis of the colloids. Since in the soil the action is always due to a combination of electrolytes, it will be of interest to study the effect of a few such combinations. Table 22 and 23 show the influence of the presence of actively charging electrolytes, such as NaOH and $\text{Na}_4\text{Fe}(\text{CN})_6$, on the flocculating, and the discharging action of $\text{Ca}(\text{OH})_2$, of CaCl_2 , and of methylene blue. The tables should be compared with table 19 and 20, which show the action of the same electrolytes on the Sharkey soil colloid without the

TABLE 21
The influence of NaOH on electrodialysed and Ca-saturated Sharkey colloid
(0.4 gm. colloid per liter)

| NaOH MILLIEQUIVALENTS IN LITER | FLOCCULATION | $\mu/\text{SEC.}$ 1 VOLT/CM. | P.D. MILLIVOLTS |
|-----------------------------------|--------------|---------------------------------|-----------------|
| <i>Electrodialysed colloid</i> | | | |
| 0.0 | 0 | -3.25 | -68.2 |
| 0.2 | 0 | -3.60 | -75.6 |
| 1.0 | 0 | -3.93 | -82.5 |
| 4.0 | 0 | -3.70 | -77.7 |
| 8.0 | 0 | -3.52 | -74.0 |
| 12.0 | 0 | -3.45 | -72.5 |
| 16.0 | 0 | -3.18 | -66.8 |
| 20.0 | xx | -3.08 | -64.7 |
| 24.0 | xxxx | -3.08 | -64.7 |
| 28.0 | xxxx | -3.05 | -64.0 |
| <i>Ca-saturated colloid</i> | | | |
| 0.0 | 0 | -1.91 | -40.0 |
| 0.2 | 0 | -3.06 | -64.2 |
| 1.0 | 0 | -3.39 | -71.1 |
| 4.0 | 0 | -3.04 | -63.8 |
| 8.0 | x | -2.81 | -59.0 |
| 12.0 | xxxx | -2.68 | -56.3 |

hydroxide and ferrocyanide of sodium. The comparison shows that the NaOH in quantities far below its own flocculating concentration, materially reduces the amount of CaCl_2 and $\text{Ca}(\text{OH})_2$ required to flocculate the *saturated* colloid, in spite of the fact that the alkali hydroxide greatly increases the cataphoretic potential. The critical potential is more than doubled by the presence of NaOH. This is in exact agreement with the author's observations made several years ago in the case of suspensions of quartz, clay, and humus (15). The comparison must only be made with the saturated colloids. The unsaturated colloid is more sensitive to the neutral salts alone, for the reasons explained. The suppressing effect of hydroxides on the flocculating power of salts, which

TABLE 22

The influence of NaOH on the action of CaCl_2 and of $\text{Ca}(\text{OH})_2$ on the Na-saturated Sharkey colloid

(0.4 gm. colloid per liter)

| CaCl_2 MILLIEQUIVALENTS IN LITER | NaOH MILLIEQUIVALENTS IN LITER | FLOCCULATION | $\mu/\text{SEC.}$ 1 VOLT/CM. | P.D. MILLIVOLTS |
|---|--------------------------------------|--------------|---------------------------------|-----------------|
| 0.0 | 2.0 | 0 | -3.86 | -81.0 |
| 0.4 | 2.0 | 0 | -3.01 | -63.2 |
| 0.6 | 2.0 | XX | -2.42 | -51.8 |
| 0.8 | 2.0 | XXXX | -2.13 | -44.7 |
| 1.0 | 2.0 | XXXX | -1.58 | -33.2 |
| <hr/> | | | | |
| $\text{Ca}(\text{OH})_2$ | | | | |
| 0.0 | 2.0 | 0 | -3.88 | -81.5 |
| 0.4 | 2.0 | 0 | -3.08 | -64.7 |
| 0.6 | 2.0 | XXXX | -2.49 | -52.3 |
| 0.8 | 2.0 | XXXX | -1.95 | -41.0 |
| 1.0 | 2.0 | XXXX | -1.50 | -31.5 |

TABLE 23

The influence of $\text{Na}_4\text{Fe}(\text{CN})_6$ on the action of CaCl_2 and of methylene blue on the Na-saturated Sharkey colloid

(0.2 gm. colloid per liter)

| CaCl_2 MILLIEQUIVALENTS IN LITER | $\text{Na}_4\text{Fe}(\text{CN})_6$ MILLIEQUIVALENTS IN LITER | FLOCCULATION | $\mu/\text{SEC.}$ 1 VOLT/CM. | P.D. MILLIVOLTS |
|---|---|--------------|---------------------------------|-----------------|
| 0.0 | 2.0 | 0 | -3.92 | -82.3 |
| 0.4 | 2.0 | 0 | -3.11 | -65.3 |
| 0.8 | 2.0 | 0 | -2.55 | -53.6 |
| 1.0 | 2.0 | 0 | -2.44 | -51.2 |
| 1.2 | 2.0 | 0 | -2.12 | -44.5 |
| 1.6 | 2.0 | XX | -1.86 | -39.1 |
| 2.0 | 2.0 | XXXX | -1.73 | -36.3 |
| 2.4 | 2.0 | XXXX | -1.66 | -34.8 |
| <hr/> | | | | |
| Methylene blue | | | | |
| 0 | 1.5 | 0 | -4.05 | -85.0 |
| 0.080 | 1.5 | XXXX | -3.20 | -67.2 |
| 0.107 | 1.5 | XXXX | -3.20 | -67.2 |
| 0.161 | 1.5 | XXXX | -2.15 | -65.8 |
| 0.268 | 1.5 | XXXX | -1.05 | -22.1 |
| 0.537 | 1.5 | XXXX | -0.28 | -5.9 |
| 0.805 | 1.5 | XXXX | ± 0.0 | ± 0.0 |

has often been observed, is evidently not due to the presence of the base, as assumed, but to the absence of the more powerfully acting products of the exchange acidity.

The presence of the ferrocyanide caused a slight suppression in the flocculat-

ing power of CaCl_2 , whereas the critical potential was nearly doubled. In the case of the methylene blue, its flocculating power was at least not suppressed by the presence of the tetravalent anion but the tetravalent anion more than doubled the critical potential. The fact, previously alluded to, that the presence of polyvalent anions causes an increase in the quantity of methylene blue required to neutralize the negative charge of the colloid, is here strikingly illustrated. About six times more methylene blue was required for the electrical neutralization in the presence of the tetravalent anion. The same effect of this ion was observed in the preparation of isoelectric alumino-silicates. The proportion of electropositive alumina required to neutralize electrically the negative silica was greatly increased by the presence of a small quantity of ferrocyanide (20). This phenomenon is being investigated in connection with a study of various isoelectric systems and will be discussed in a later publication.

THE EFFECTS OF ACIDS

Negative and Positive Soil Colloids

Because of the amphoteric behavior of soil colloids which contain a high proportion of sesquioxides, the influence of acids may be very different in different colloids, depending upon the composition of the colloids. The author has already shown how cataphoresis in acid solutions is related to the silica/sesquioxide ratio (19). It has also been shown that those colloids which are charged electropositively in acid solutions adsorb appreciable quantities of the anions of the acid. The order of magnitude of the adsorption is:



Of these ions only the phosphate is adsorbed from neutral and alkaline solutions but the adsorption is much greater in acid solutions. The adsorption of all the anions mentioned, increases progressively with an increase of sesquioxide content of the colloid. The relationship between the positive charge and the anion adsorption was explained by assuming the formation of compounds like oxy-chlorides, which dissociate into diffusible anions and an electropositive colloidal complex.

It was found that the magnitude of the positive charge bore no relation to the quantity of anions adsorbed, which was always greatest in the case of the PO_4 ion and least in the case of the Cl . The charge was very much greater in the HCl than in the H_2SO_4 solution. The H_3PO_4 , which at low pH values dissociates only one H, acted more like HCl . The influence of these acids upon the amphoteric soil colloids therefore resembles closely their influence upon the proteins, as observed by Loeb. It is evident that the charge depends to a great extent upon the valence.

The influence of the aforementioned three acids on the cataphoresis of two colloids very different in composition is shown in tables 24 and 25. The Mar-

shall silt loam colloid, which has a silica/sesquioxide ratio of 2.82, is strictly electronegative, whereas the Aragon clay subsoil colloid, with a ratio of 0.55, is amphoteric in behavior (19). Since, in the case of the Aragon colloid,

TABLE 24
Cataphoresis of the Marshall soil colloid in HCl, H₂SO₄, and H₃PO₄ solutions
(0.2 gm. colloid per liter)

| MILLIEQUIVA- LENTS IN LITER | HCl | | H ₂ SO ₄ | | H ₃ PO ₄ | |
|--------------------------------|---------------------------|--------------------|--------------------------------|--------------------|--------------------------------|--------------------|
| | μ /sec. 1 volt/cm. | P.D. millivolts | μ /sec. 1 volt/cm. | P.D. millivolts | μ /sec. 1 volt/cm. | P.D. millivolts |
| 0.0 | -2.35 | -48.3 | | | | |
| 0.1 | -1.85 | -38.8 | -2.05 | -43.0 | -1.91 | -40.0 |
| 0.4 | -1.83 | -38.4 | -2.20 | -46.2 | -1.76 | -37.0 |
| 1.0 | -1.75 | -36.8 | -2.25 | -47.2 | -1.63 | -34.2 |
| 4.0 | -1.55 | -32.5 | -1.91 | -40.0 | -1.65 | -34.6 |

TABLE 25
Cataphoresis of the Aragon soil colloid in HCl, H₂SO₄, and H₃PO₄ solutions
(0.2 gm. colloid per liter)

| MILLIEQUIVALENTS IN LITER | pH | FLOCCULATION | μ /SEC. 1 VOLT/CM. | P.D. MILLIVOLTS |
|--------------------------------|------|--------------|---------------------------|-----------------|
| HCl | | | | |
| 0 | | xx | -1.07 | -22.5 |
| 0.01 | 6.10 | xxxx | +0.32 | +6.7 |
| 0.1 | 4.53 | xxxx | +1.58 | +33.2 |
| 1.0 | 3.25 | xxxx | +1.90 | +39.9 |
| 4.0 | 2.55 | xxxx | +2.18 | +45.8 |
| H ₂ SO ₄ | | | | |
| 0.01 | 6.25 | xxxx | +0.10 | +2.1 |
| 0.1 | 4.40 | xxxx | +0.73 | +15.3 |
| 1.0 | 3.25 | xxxx | +0.39 | +8.2 |
| 4.0 | 2.59 | xxxx | +0.23 | +4.8 |
| H ₃ PO ₄ | | | | |
| 0.03 | 6.05 | 0 | -1.26 | -26.4 |
| 0.3 | 4.47 | xxxx | ± 0.0 | ± 0.0 |
| 3.0 | 3.28 | xxxx | +0.68 | +14.3 |
| 12.0 | 2.70 | xxxx | +0.63 | +13.2 |

adsorption of the acids was considerable, the pH values were determined by the quinhydrone electrode. This was done after three days standing.

In the case of the Marshall soil colloid there is no great reduction in the negative charge, and the suppressing effect of the acids would undoubtedly be even smaller if it were not for the formation of secondary products. Quartz

particles increase their speed of migration in a dilute HCl solution. The valence effect of the anions is unmistakable and it is interesting to note that the phosphoric acid suppresses the charge fully as much as the hydrochloric, whereas the sulfuric acid alone exerts a weaker suppression. This is, as pointed out by Loeb, because free H_3PO_4 dissociates chiefly the monovalent anion H_2PO_4^- . In neutral sodium phosphate the charge of the particles is considerably increased. The maximum speed of migration of the Marshall particles in solutions of KCl, K_2SO_4 , and neutral sodium phosphate was found to be 2.8, 3.0, and 4.0μ per second respectively.

The effect of the three acids on the Aragon colloid is very different. This colloid, which has a very high sesquioxide content, is charged electropositively in a carbonic acid solution. In freshly distilled water the particles migrated toward the anode with an average velocity of 1.07μ per second. In old CO_2 -contaminated water the material was about isoelectric. A concentration of 0.01 milliequivalent of HCl and H_2SO_4 per liter was sufficient to change the sign of charge but, whereas the positive charge is progressively increased by the HCl over the entire concentration range, it soon reaches a maximum in the H_2SO_4 solution beyond which the positive charge is suppressed by the free, divalent anion. The valence effect on the positive side acts in the opposite direction to that on the negative side of the isoelectric point, the divalent anion causing the greatest suppression of the positive charge.

The effect of the H_3PO_4 shows an interesting deviation. The lowest concentration (0.01 millimol) charges the particles more electronegatively and brings about a decided peptisation, as was shown by lasting turbidity in this case whereas all the other acidified suspensions settled clear over night. Even the water suspension flocculated about half completely. (The experiment was repeated with the same results.) The next higher H_3PO_4 concentration rendered the colloid isoelectric, whereas the two highest concentrations rendered the particles more strongly electropositive than in the case of the corresponding H_2SO_4 concentrations. This is evidently because the monovalent di-hydrogen phosphate ion suppresses the positive charge less strongly than does the divalent SO_4 ion.

The initial elevation of the negative charge by the H_3PO_4 might be assumed to be due to the formation of phosphates yielding divalent and possibly trivalent anions. But just why these ions should increase the cataphoretic potential is, in view of the above observations, not so easy to explain. The phosphate ion is adsorbed by all soil colloids under all conditions of reaction and, according to the generally accepted view, an adsorbed anion should increase the negative charge. But the alkali sulfates, and even more strongly the ferrocyanides, increase the negative charge of negative colloids, and yet these anions are apparently not positively adsorbed, but are, on the contrary, negatively adsorbed because of Donnan distribution. In acid solutions, the Cl and the SO_4 ions are both adsorbed to an appreciable extent by soil colloids with a high sesquioxide content. But this adsorption of the anions, instead of

increasing the negative charge, brings about its destruction and imparts a positive charge to the colloid. The magnitude of this positive charge bears however, no relation to the quantity adsorbed. The Cl ion which is least adsorbed imparts the strongest positive charge.

This relationship between the anion adsorption and the positive charge is satisfactorily accounted for by the formation of basic salt complexes by an interaction between the sesquioxides and the acids. A dissociation of these combinations results in an atmosphere of anions surrounding an electropositive particle just as the electronegative particle is surrounded by an atmosphere of cations. The magnitude of the charge must depend, among other factors to be mentioned later, on the degree of dissociation, i.e., on the number of free charges per unit surface. It is in this connection that the specific nature of the ions plays an important rôle and gives rise to the Hofmeister or lyotropic ion series.

On the basis of this theory the increase in charge resulting from the addition of certain electrolytes might be explained. If the micellar ion is different from the corresponding ion in the added electrolyte an exchange is known to take place. If the ingoing ion remains dissociated to a greater extent than the outgoing ion an increase in charge must follow, provided that the suppressing effect of the free electrolyte is not great enough to counteract the increase, as will always be the case in higher concentrations. If we look at table 18 we will find that the KCl increased the charge in low concentrations only in the case of the original (chiefly Ca- and Mg-saturated) and the Ca-saturated colloids but not in the case of the H- and Na- saturated colloids. In table 21 we see that NaOH increased the charge in the case of the H-saturated as well as in the case of the Ca-saturated colloid. In other words the charge, as measured by cataphoresis, is increased whenever the displacing ion gives rise to a more highly dissociated complex. When the displacing ion is of the more highly associating type such as the divalent ions and the methylene blue ion, then the charge is reduced at the outset. (The aforementioned anomaly of bentonite will be discussed later.)

Effect of salts on positive colloids

Before any work had been done on the negative adsorption or Donnan distribution in the case of electropositive soil colloid gels, it was concluded from the cataphoresis experiment with the Aragon colloid in acid solutions that the Cl ion is more highly dissociated by the particles than is the SO_4 ion. If this were the case then the addition of a chloride to the sulfated complex of the colloid should increase the positive charge just as the addition of an alkali salt increases the negative charge of the Ca-saturated colloid. It has already been pointed out that the adsorbed anions displace one another (19). But since the different anions are "adsorbed" in very different proportions their exchange differs radically from base exchange which takes place in equivalent proportions. And just as an alkali sulfate imparts a stronger negative charge than an alkali chloride to the electronegative Ca-saturated colloid because of the weaker

suppressing influence of the divalent anion of the free electrolyte, so, for the same reasons, but in a reversed sense, must a chloride with a divalent cation permit a greater increase in the positive charge of the electropositive SO_4 -complex. On the other hand, the addition of sulfates and, even more so, the addition of ferrocyanides must suppress the positive charge at the outset.

The experiment, shown in table 26, fully confirmed the predictions.

The chlorides did not greatly increase the positive charge, but it must be remembered that an increase due to the formation of a more highly ionized Cl -complex is opposed at the outset by the suppressing influence of the free electrolyte. The suppression is greatest in the case of NaCl and weakest in the case of CaCl_2 because the ion of the same sign of charge as the colloid, i.e., the cation, is divalent in the latter salt. (Compare table 2, part I.)

TABLE 26
Influence of salts on the electro-positive SO_4 -complex of the Aragon colloid
(0.2 milliequivalents H_2SO_4 and 0.2 gm. colloid per liter)

| SALT MILLIEQUIVALENTS IN LITER | FLOCCULATION | $\mu/\text{SEC.}$ 1 VOLT/CM. | P.D. MILLIVOLTS |
|-------------------------------------|--------------|---------------------------------|-----------------|
| 0 | XXXX | +0.89 | +18.7 |
| NaCl | | | |
| 0.2 | XXXX | +0.94 | +19.7 |
| 1.0 | XXXX | +1.05 | +22.1 |
| CaCl_2 | | | |
| 0.2 | XXXX | +1.08 | +22.7 |
| 1.0 | XXXX | +1.16 | +24.4 |
| Na_2SO_4 | | | |
| 0.2 | XXXX | +0.72 | +15.1 |
| 1.0 | XXXX | +0.42 | +8.8 |
| $\text{Na}_4\text{Fe}(\text{CN})_6$ | | | |
| 0.2 | XX | -0.64 | -13.4 |
| 1.0 | X | -1.89 | -39.7 |

The sulfate exerts only a suppressing effect on the charge of the sulfated complex. This is similar to the action of CaCl_2 on the Ca -saturated complex.

The ferrocyanide not only suppresses the positive charge but renders the colloid electronegative. This is to some slight extent due to a suppression of the hydrogen-ion concentration, but the pH was appreciably displaced only in the higher ferrocyanide concentration. It was low enough (about 4.5) even here however, to impart a positive charge in the absence of the salt. As far as the phenomenon may be related to the Donnan equilibrium the effect can only be in the form of a suppression. The suppression must in this case be very great because of the four valences of the anion. But the Donnan distribution of the free electrolyte can only exert a discharging effect; it cannot increase the charge nor change its sign. We may conclude that the di- and trivalent phosphate

ions and the tetravalent ferrocyanide ion form a nondissociated complex with the soil material. The particles must then assume a negative charge by the same mechanism by which all other materials charge themselves electronegatively in aqueous suspension. The electropositive charge is apparently confined to a special condition of matter, by virtue of which diffusible anions are dissociated by the dispersed phase. The electronegative behavior of materials dispersed in water appears to represent a more general condition. A conception of how this may be related to the structure and properties of the interfacial layer of water has been presented in previous papers (20, 21).

Regarding the stability of the Aragon colloid we find again no definite relationship to the cataphoretic potential. While the lowest H_3PO_4 concentration and the ferrocyanide caused a distinct peptisation, all the other suspensions were completely flocculated although some of them showed a higher potential or, to speak more cautiously, a higher migration velocity.

It should be pointed out that the migration velocity may not be directly proportional to the cataphoretic potential, as would be the case if all the other factors in the Helmholtz-Perrin formula remain constant. But we are hardly justified in assuming values for the viscosity and the dielectric constant equal to that of ordinary "free" water. The water molecules or hydrols within the micellar atmosphere must, insofar as they are electrical bipoles, occupy an oriented position. This means that "adsorbed" water is structurally different from "free" water. The oriented hydrols are under the influence of an electrical moment and therefore must offer a greater resistance to a displacement, as in cataphoresis, than would be offered by free hydrols distributed at random. A greater viscosity within the double layer may therefore be assumed. The addition of electrolytes may diminish, in various degrees, the electrical moment and the orientation, resulting in a decreased viscosity. Electrolytes are known to diminish the dielectric constant. It is therefore evident that there can be no exact proportionality between cataphoresis and the P.D. of the double layer. Nevertheless, the migration velocities at the point of flocculation differ so greatly (and are in certain well-defined cases even greater at this point than before the electrolyte is added) as to make it very doubtful that flocculation takes place at the same P.D. in every case. In the case of colloid, oil drops, and other inert materials the critical potential may be very nearly the same for all electrolytes, but in the case of soil particles which are surrounded by a dense atmosphere of ions and a comparatively thick layer of osmotically imbibed water, the critical potential seems to vary with the nature of the ions. This indicates the existence of other stability factors.

FACTORS OF STABILITY

Dissociation

The idea that flocculation is caused by an adsorption of the ions of opposite sign of charge which neutralize the free charges on the surface of the particles

and thereby suppress the cataphoretic potential, does not provide us with an adequate explanation. It has been shown that the ions of the same sign of charge as the colloid influence flocculation according to their valence and that this is true even when there is no adsorption of the ions. Given (9) and later Mattson (15) found the same influence in the case of different calcium salts, and it was significantly observed by P. Ehrenberg (7, p. 303) that the SO_4 ion was not adsorbed by the clay although it had been found that the ion did influence the flocculation. If the observation made in part I of this paper, that the dissociation of the colloid complex, i.e., the value of z , is increased by the addition of electrolytes, is substantiated, then, neither discharge nor flocculation can be the result of adsorption or a suppression of ionization of the ion of opposite sign of charge, and our theory will call for a radical revision.

An increase in dissociation of the complex with an increase in the added electrolyte is not so absurd as it might at first seem. If the base is adsorbed by virtue of a fixation of the OH ions at the interface then the cations are in reality not by themselves adsorbed but are merely attracted by the ions of opposite sign at the interface. Only a few of the cations are able to diffuse into the external medium because a critical potential is established against which the ions cannot dissociate. This limiting potential appears to be equal to that of the common ions, or about 70 millivolts. As far as formula (C) (see part I) is applicable, the author (17) calculated that in the case of a colloidal particle with a radius of $45.5\mu\mu$ and containing 505,208 exchangeable cations, a dissociation of only 857 ions would account for the cataphoretic potential. In the absence of other electrolytes a very slight dissociation is apparently sufficient to raise the potential to the limiting value. The addition of electrolytes suppresses the potential, thus weakening the electrostatic attraction on the cations and causing a greater number of them to diffuse into the outer medium. This explains the constancy of the product (P.D.) z which seems to prove the connection between dissociation and the potential. Thus by looking upon the colloidal complex as an adsorption ionogen which holds one of the ions (that of opposite charge) merely to preserve a certain electrical balance, it becomes comprehensible why a suppression of the P.D. leads to a greater dissociation.

Activity

The preceding phenomenon is similar to the "salt effect" on weak electrolytes as observed in connection with the indicator method and described by Brönsted (1a) as an expression of a change in the activity or potential of the ions. The interionic attraction theory of activity as formulated by Debye and Hückel (5) to explain the deviations of strong electrolytes from the ideal gas laws has much in common with our conception of the colloidal micelle and might successfully be applied to the problem here dealt with. This theory takes into account the mutual attraction between the ions of opposite charge. This attraction leads to the condition that in the neighborhood of an ion there is on the average more ions of unlike than of like sign of charge. The result

of this interionic action is a suppression of the potential, or activity, of the individual ions. When two ions of unlike sign are a great distance apart, as in very dilute solutions, their potential or activity approaches a maximum. As two such ions are brought closer together, their potentials are progressively suppressed as their fields overlap. An actual contact or association of the ions results in a zero potential, except in so far as a residual field remains, because of a polarization from an asymmetry in the molecule. Since the Coulomb forces between the ions are inversely proportional to the square of the distance between them, it follows that the suppression of the self-potential or activity of the ions must be proportional to the square root of the concentration. Thus the molar conductivities of strong electrolytes decrease with an increase in concentration according to the well-known Kohlrausch's square-root law.

This, in common language, may serve to illustrate the fundamental concept in the interionic attraction theory. The mathematical formulation of the theory has led to the following simple limiting equation applicable to dilute solutions

$$-\log \gamma_s = 0.5 Z_a Z_b \sqrt{\mu}$$

where γ_s is the mean activity coefficient of the ions a and b of a compound, Z_a and Z_b the valence of the ions, and μ the ionic strength of the solution. $\mu = \frac{1}{2} \sum m_i z_i^2 = \frac{1}{2} \sum c_i z_i^2$ where m is molality, c equivalent concentration, and z valence of any ion present in the solution (13).

Thus if in a solution of a weak acid $H A$, μ is increased by the addition of a salt, then the activity coefficient of the ions will be diminished in proportion to $\sqrt{\mu}$ while the activity coefficient of the neutral molecules will be unaffected. This leads to an increase in dissociation in accordance to the expression

$$\frac{\gamma_{H^+} \gamma_{A^-}}{\gamma_{HA}} = K_\gamma$$

in which K_γ remains a true constant which would of course not be the case in the classical mass action expression

$$\frac{C_{H^+} C_{A^-}}{C_{HA}} = K_c$$

in which the factors represent concentration instead of activity.

Similarly in the case of a slightly soluble salt an increase in μ by the addition of an extraneous salt will result in a greater solubility as the activity coefficient of the ions is diminished. In the presence of the solid phase the concentration of the dissolved salt multiplied by the activity coefficient should remain constant, thus

$$[\text{salt}]_1, \gamma_1, = [\text{salt}]_2 \gamma_2 \text{ etc.}$$

where the brackets signify concentration.

By introducing the above equation we get

$$\log \frac{[\text{salt}]_1}{[\text{salt}]_2} = \log \gamma_2 - \log \gamma_1 = 0.5 Z_a Z_b (\sqrt{\mu_1} - \sqrt{\mu_2})$$

which shows the logarithm of the ratio of the two solubilities of the salt to be a linear function of the square root of the ionic strength of the solution. For applications of this theory the reader is referred to Börnsted and LaMer (2, 29), Noyes (24), Müller (23), and to Clark (4, chap. XXV).

The foregoing illustrates the operation of the square root law as far as this factor alone affects the activities of the ions of a given compound under study. In the preceding equation there is another factor which, for the problem here dealt with, is of fundamental importance and deserves attention. This is the influence of the valence of the ions of the compound affected, which is expressed by the valence factor $Z_a Z_b$. From this it is evident that the suppression of the activity coefficient will increase with the valence of the ions of the compound, the suppression being proportional to the product of the valence of the two ions. The coefficient $0.5 Z_a Z_b = 0.5$ for a uni-univalent salt; for a uni-bivalent it is $= 1$; for a uni-trivalent it is $= 1.5$; for a tri-trivalent it is $= 4.5$. Now it becomes at once evident that, as far as the equation is at all applicable to colloidal ionogens, one of the ions of which is greatly multivalent, the suppressing effect of an added salt on the activity or potential of the ions will be very much greater as compared to the effect on ordinary electrolytes. This will explain why the value of z , i.e., the concentration in the micellar atmosphere of the ions dissociated by the colloid, was found to increase so rapidly with an increase in the amount of salt added. The value of z increased nearly ten-fold between a salt concentration of 0.005 N to 0.4 N (see table 3).

Dissociation and ionic strength

In view of what has been said in the foregoing, the significance of a relationship, not before mentioned, will readily be recognized. If the z values as ordinates are plotted against the square roots of the corresponding y concentrations, as in figure 4, a straight line is obtained which, when extrapolated, passes through a point very near the origin. The values are taken from tables 3 and 7, part I. Here \sqrt{y} is equal to $\sqrt{\mu}$, since the added salt (NaCl) is uni-univalent.

The linear relation shows: first, a connection between the dissociation of the colloid and the square root of the ionic strength; and, second, that the colloid is only very slightly dissociated in the absence of an extraneous electrolyte.

It follows from purely electrokinetic considerations that a highly multivalent colloidal complex cannot dissociate to the same extent as common salts. The latter dissociate into ions with only one, two, or three, electronic charges. The potential and the resulting attraction between these ions are never greater than can be overcome by the force of thermal agitation and the tendency to disperse. In the case of very small ions in which the self potential would be too high the

stability of the ions is increased by an association with the molecules of the solvent. Common salts may therefore be completely dissociated.

A colloidal ionogen, on the other hand, can only dissociate to a point at which a limiting potential is established. At this point the force of thermal agitation is balanced by the electrostatic attraction. In the absence of extraneous electrolytes, when the activity coefficient is at a maximum this condition is apparently attained at a comparatively low degree of dissociation. If a soil colloidal particle with a radius of $45.5 \mu\mu$ and containing 505,208 exchangeable cations were completely dissociated, the ion density would be extremely great, resulting

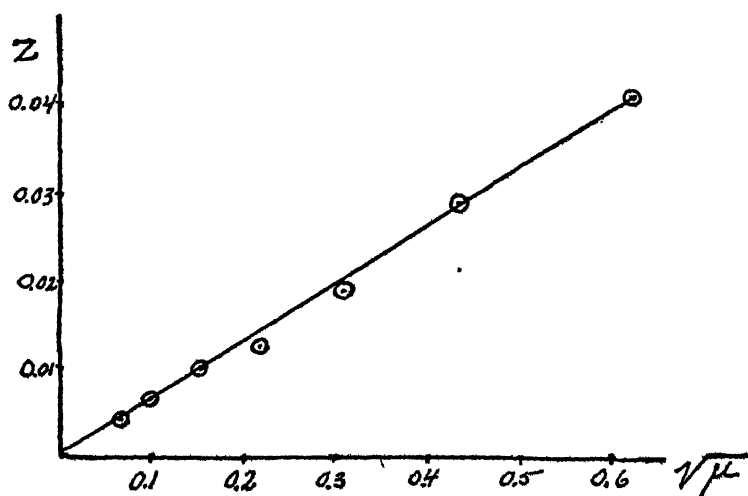


FIG. 4. THE LINEAR RELATION BETWEEN THE DISSOCIATION OF THE COLLOIDAL COMPLEX (VALUES OF Z) AND THE SQUARE ROOT OF THE IONIC STRENGTH OF THE SOLUTION

in a potential several hundred times as great as has ever been observed. Applying equation (C) part I,

$$\text{P.D.} = \frac{e \delta}{Dr (r + \delta)}$$

and assuming a mean thickness of the double layer $\delta = 5 \mu\mu = 5 \times 10^{-7}$ cm. yields a potential of 19.6 volts. This is nearly 300 times as great as the usually found maximum of 70 millivolts. It is obvious that a potential of this order of magnitude would powerfully attract the dissociated ions, leading to an association with the interfacial layer of ions of opposite sign of charge. The assumed thickness of the double layer of $5 \mu\mu$ was certainly taken low enough in view of the preceding observed thickness of the ion atmosphere (see part I) but even if we assume a thickness of molecular dimensions, which would be absurd, the calculated potential would still be much too high.

Since the value of z , or the number of free charges per unit surface, increases with the square root of the ionic strength, it is obvious that equation (C) cannot be applied in its present form in the presence of free electrolytes any more than the ordinary mass action law can be applied. In this equation a constant value for each electronic charge e is assumed but this must vary with the activity coefficient of the ions. For values smaller than unity the latter should necessarily be introduced in the equation thus

$$\text{P.D.} = \frac{e \gamma \vartheta}{Dr (r + \vartheta)}$$

Dissociation of proteins

In his study of the proteins, Loeb (14) assumed a complete dissociation of the protein hydrochloride combinations. A careful examination of Loeb's data

TABLE 27
Percentage of dissociation of gelatin chloride calculated from Loeb's tables

| M.EQ. HCl ADDED TO 100 CC. GELATIN SOLUTION | M.EQ. HCl ADDED TO 350 CC. OUTSIDE SOLUTION | TOTAL M.EQ. HCl ADDED | Cl ACCOUNTED FOR BY $x+y+z$ M.EQ. | DIFFERENCE = NONDISSOCI- ATED Cl M.EQ. | DISSOCIATED = z M.EQ. | PER CENT DISSOCIATION |
|--|--|--------------------------|--|--|----------------------------|--------------------------|
| 0.1 | 0.0095† | 0.1095 | 0.0448 | 0.0647 | 0.0165 | 20.3 |
| 0.2 | 0.0221* | 0.2221 | 0.1123 | 0.1098 | 0.0514 | 31.9 |
| 0.6 | 0.136* | 0.736 | 0.4061 | 0.3299 | 0.200 | 37.8 |
| 0.8 | 0.298* | 1.098 | 0.8618 | 0.2362 | 0.343 | 59.3 |
| 1.0 | 0.581* | 1.581 | 0.9702 | 0.6108 | 0.372 | 38.0 |
| 1.5 | 1.79* | 3.29 | 2.740 | 0.55 | 0.608 | 52.6 |
| 2.0 | 3.04* | 5.04 | 4.850 | 0.154 | 0.609 | 80.0 |

* Calculated from the pH values in column 4 table 1. All the other figures in this table are calculated directly from the corresponding values in tables 11 and 14 in the work referred to.

† Based on a pH value = y . A pH value = x would give 17.0 per cent dissociation. The true percentage dissociation should therefore be between 17.0 and 20.3 per cent.

reveals that this ~~this~~ is not the case as far as the value of z , as calculated from the equilibrium equation, can be relied upon as an index of dissociation.

Loeb added HCl in increasing quantities to 100-cc. 1 per cent solutions of originally isoelectric gelatin contained in collodion bags, and immersed these bags in 350-cc. HCl solutions brought to the same pH as that of the gelatin solution inside the bags. By adding together the values of x , y , and z in terms of total milliequivalents in the outside and in side solutions at equilibrium and subtracting this sum from the total HCl added (a) to the 100-cc. gelatin solution and (b) to the 350-cc. outside solution, a difference was obtained for each concentration and, what is still more significant, the differences are relatively greater in low than in high concentrations. This means that the protein chloride is less dissociated in dilute than in more concentrated HCl solutions. This is contrary to the idea of a suppression of ionization by the

presence of a common ion but it is in harmony with the activity concept as applied, in the foregoing, to multivalent complexes.

The results of this calculation are given in table 27, the data being obtained from Loeb's tables 14, 11, and 1.

It will be seen that the calculated dissociation increases from about 20 to 80 per cent as the concentration of the free acid is increased. The increase, however, is, for some reason irregular. In the case of bentonite the dissociation increased from about 5 to 25 per cent as the concentration of NaCl was increased from 0.005 *N* to 0.4 *N*. The gelatin chloride is therefore more highly dissociated, although the Donnan P.D. is very nearly the same in the case of both materials at the same free electrolyte concentration. (Compare, for example, the action of NaNO₃ in Loeb's table 18 with the author's table 4 in part I.) The maximum quantity of HCl in combination with the gelatin is approximately the same as the quantity of base in combination with the saturated bentonite. The gelatin complex is therefore absolutely, as well as relatively, more dissociated than the bentonite complex. The nearly identical P.D. values, in spite of this difference in dissociation, are, of course, explained by the different concentrations used, i.e., a 1 per cent gelatin "solution" as compared to a 10 per cent bentonite gel.

The fact that the gelatin complex can dissociate to a greater extent than the bentonite complex without exceeding the critical potential must be due to the greater dispersion and especially to the greater hydration of the gelatin complex. A greater dispersion reduces the ion density to the unit surface, thus permitting a higher degree of dissociation. The probable effect of an extensive association with the water molecules must be a suppression of the potential, because these molecules, if polarized, are not neutral, and the cause of the association must itself depend upon the attraction between poles of unlike sign. Association with the water will therefore permit the complex to dissociate more ions. This is merely an application of a principle known to exist in the case of the common ions. Thus the self potential or activity of ions like Li or Na is suppressed by association with water molecules. We have already seen how a dehydration, e.g., by the addition of alcohol, increases the activity of these ions, which increase shows itself in a lower dissociation of the colloid complex (compare table 11). Further, it is well known that although the activity coefficient decreases in low to moderate concentrations, it again increases rapidly in high concentrations, and that the higher the hydration value of the ions is the greater is the augmenting effect in high concentrations (27). Thus the addition of large quantities of LiCl to a solution will have a greater effect upon the activity of the ions in solution than an equal quantity of NaCl or KCl because the Li ions, being more highly hydrated, compete more strongly for the water. This explains also why, in very strong solutions of the various chlorides, the difference in displacing power between the cations tends to vanish.

An increase in the dissociation of gelatin chloride with an increase in the concentration of the free electrolyte is also evident in the experiments of Loeb

where he studied the influence of neutral salts. These experiments, in which the HCl concentration was kept constant, are more comparable to the preceding experiments with the soil colloids, but, since Loeb studied the ion distribution by measuring the pH and does not give any data on the distribution of the ions of the salt, which is necessary for the calculation of z , the relationship between dissociation and the ionic strength is not so apparent. But a test calculation will show that if z remained the same, "neglecting the diminution of ionization of gelatin chloride" (14, p. 202), the suppressing effect of the salt would be much greater than it was found to be. In the experiment with NaNO_3 (14, table 18) the value of z equals 0.00275 N in the absence of salt. In the presence of .051625 M NaNO_3 , z must be equal to 0.0060 N to account for the reported P.D. value. In other words the dissociation has increased instead of diminished by the addition of the salt.

The increase in concentration weakens the activity of the ions and thus reduces the energy content of the solution. In view of the fact that the equilibrium between the undissociated complex and its ions must be an energy equilibrium rather than a mass equilibrium, it is easily understood that this effect of the salt will be a displacement of the equilibrium in the direction of increased dissociation. Thus the potential of a colloid particle or of a gel, being suppressed by the addition of an electrolyte, strives to maintain itself. This, within certain limits, is possible through a further dissociation. We may speak, therefore, of the colloid as being electrically buffered.

It thus appears that the observed increase in dissociation of the colloidal complex following an increase in salt concentration can be related to theoretically well-founded principles. The same phenomenon is encountered in the case of the simple ionogens but there is a quantitative difference between the two groups in that the effect is greatly intensified in the case of the colloid ionogen because of its highly multivalent character.

Present views on charge and adsorption

The generally accepted idea that the suppression of the interfacial potential and of the stability is due to a neutralization of the free charges by the adsorption of ions of unlike sign is, in view of the foregoing, untenable. It is true that such ions are adsorbed, but usually only by an equivalent exchange. A diminution in the potential could then, from this point of view, only result when the ingoing ion is less dissociated by the complex than the outgoing ion. It would be difficult to explain the flocculation of the Ca-saturated colloid by a sodium salt. Krøyt, who admits the equivalence of exchange, seems to forget the roll of the H ions already present in the As_2S_3 -complex, which he discusses when he says, "For the removal of the electric charge originally present, there is required, of course, a definite amount of positive electricity, or, in other words, a definite number of ions" and "that equivalent amounts of ions of different valence must be adsorbed in order to produce the same lowering of the interfacial potential" (11, p. 74). When the added electrolyte has the same cation

as the colloid there is no exchange and usually, therefore, no adsorption, yet flocculation will take place just as readily. A neutralization of the charge by adsorption would, in this case, be conceivable only if the added electrolyte suppressed the dissociation of the colloidal complex. But since the very opposite effect has been established, it is obvious that this theory cannot be accepted.

The net results of the foregoing may be summarized as follows:

The stability of the colloid depends upon the degree of dissociation of the complex, which in turn depends upon the nature of the ions of opposite sign of charge. The addition of an electrolyte results (a) in the formation, by exchange, of a new complex which may differ in stability from that of the original; (b) in a diminution in the activity, i.e., in the self-potential of all ions resulting; (c) in a higher degree of dissociation of the complex; (d) in a suppression in the P.D. between the micellar and intermicellar solutions according to the Donnan equation; and (e) in a suppression of the excess osmotic pressure within the micellar solution, resulting in a diminution in the osmotic hydration of the micelles.

How largely each of these and other, as yet unknown, changes contribute to the conversion of a stable sol into a coagulated mass is, at our present stage of knowledge, impossible to say. We know as yet too little about interfacial structure and interfacial forces. Interionic attraction, ionic association, molecular orientation, changes in dielectric constant, hydration, etc., all of which have been discussed in the foregoing, must be more fully understood before a successful solution of the problem is possible.

Hydration

It is not at all certain that the interfacial potential is directly responsible for the stability of a colloid, as already pointed out with reference to the work of Porter and Hedges (25). The charge of the particles must, however, be indirectly connected with the stability. Thus the osmotic hydration is proportional to the degree of dissociation of the complex, and hydration certainly is a stability factor. The great stability of the lyophile colloids, which do not flocculate until dehydrated at "salting out" concentrations, is well known. Whereas the emulsoid or lyophile colloids, like agar and gelatin, adsorb water by molecular attraction, the suspensoid type of colloid, like the soil minerals, do not attract water in this manner; but like the lyophile colloids, the suspensoid colloids imbibe water osmotically, the imbibition being proportional, as has been shown, to the degree of dissociation and therefore related to the charge. Hence soil colloids having a low exchange capacity imbibe less water and are, as will be shown, less stable than the colloids with a high exchange capacity. Similarly a given soil colloid saturated with NaOH will imbibe more water and be more stable than when saturated with Ca(OH)_2 . When dehydrated with alcohol, the soil colloids remain stable, provided all free electrolytes have first been removed as in electrodialysis, but become in this condition extremely sensitive to flocculating agents.

Table 28 shows the action of NaCl and KCl on Na- and K-saturated Norfolk and Sharkey soil colloids, respectively, in aqueous and in 75 per cent (by volume) alcoholic suspensions.

Table 28 yields the following information:

1. The lower the exchange capacity and the lower therefore the number of cations dissociated, resulting in a lower osmotic imbibition, (Comp. table 14), the greater is the sensitivity of the colloid to the flocculating action of the electrolyte. Thus the Norfolk colloid is flocculated in concentrations about ten times lower than is required by the Sharkey colloid. 2. The lyotropic effect is very great, as shown by the influence of the different cations. Thus the colloid which is saturated with the more hydrated and more extensively dissociating Na ions and which carries therefore a thicker envelope of osmotically imbibed water is less sensitive to the action of electrolytes. Thus it requires about twice as much NaCl as KCl for complete

TABLE 28

Flocculation of Na- and K-saturated Norfolk and Sharkey soil colloids in aqueous and alcoholic suspensions by NaCl and KCl

| Na-SHARKEY | | K-SHARKEY | | Na-NORFOLK | | K-NORFOLK | |
|--|--------------|-----------|--------------|------------|--------------|-----------|--------------|
| NaCl N | Flocculation | KCl N | Flocculation | NaCl N | Flocculation | KCl N | Flocculation |
| <i>Aqueous suspensions</i> | | | | | | | |
| 0.07 | 0 | 0.025 | 0 | 0.003 | 0 | 0.002 | 0 |
| 0.08 | xx | 0.030 | x | 0.004 | x | 0.003 | xxx |
| 0.09 | xxx | 0.035 | xx | 0.005 | xx | 0.004 | xxxx |
| 0.10 | xxxx | 0.040 | xxx | 0.006 | xxx | | |
| | | 0.045 | xxxx | 0.007 | xxxx | | |
| <i>Alcoholic suspensions 75 per cent absolute by volume)</i> | | | | | | | |
| 0.0008 | 0 | 0.0004 | 0 | 0.0008 | 0 | 0.0002 | 0 |
| 0.0010 | xxx | 0.0005 | x | 0.0010 | xx | 0.0003 | xxxx |
| 0.0012 | xxxx | 0.0006 | xx | 0.0012 | xxxx | | |
| | | 0.0008 | | | | | |

Exchange capacity m.eq./gm.: Sharkey, 0.796; Norfolk, 0.207.

Silica/sesquioxide ratio: Sharkey, 3.18; Norfolk, 1.63.

flocculation of each of the aqueous suspensions. It will be noted that this explanation assigns the lyotropic effect to the cation in combination with the colloid rather than to the cation of the free salt. The ions of the free electrolyte can only exert a suppressing effect and this suppression is apparently alone governed by the concentration and valence according to the Donnan equation, as was found in the case of the nonadsorbable anions. 3. The addition of alcohol dehydrates the ions thus increasing their activity, that is, their self-potential. This leads to a diminution in the degree of dissociation and consequently also to a decrease in the osmotic imbibition, as shown in table 11. The result is a greater sensitivity of the colloids, approaching in this respect the Ca-saturated materials. The flocculating value is greatly reduced, being now around 0.001N or about that of the Ca-saturated colloid. It will also be noted that the great differences in sensitivity between the two colloids in aqueous suspensions tend to vanish in alcoholic suspensions.

It is very essential that a distinction be made between molecularly attracted water and osmotically imbibed water. It is evident that Krut, in his study of the lyotropic influence, fails, with others, to recognize the latter form of hydration when he states, "If it (the lyotropic influence) were merely a matter of hydration, one would expect the strongly hydrated lithium ion to be a better flocculating agent than the slightly hydrated potassium ion, whereas the reverse is true" (11, p. 236). In other words, we should expect the Li ion to compete more strongly for the water and remove the protecting water envelope from the micelles and thus render them more sensitive. This is actually what takes place in the salting-out process of emulsoid colloids. Here the concentration is so high that the ions have to compete for the water. The most strongly hydrated ions are therefore the most active salting-out agents. The same applies to the increase in the activity coefficient in strong solutions. In flocculation, on the other hand, the concentration is so low as to exclude any such competition for the water molecules. The Li ion is the weakest flocculating cation for the same reason that it is a strong salting-out ion. It is strongly hydrated and therefore highly dissociated by the complex, giving rise to the highest degree of osmotic hydration, thus increasing the stability of the micelles. When a lithium salt is added to the negative colloid there is an exchange of cations, and a complex partly saturated with this cation is formed which will possess a higher charge and a higher osmotic hydration. A higher concentration is therefore required to suppress this stability factor to its critical value.

The foregoing observations would seem to justify the conclusion that the osmotic hydration is a dominating factor of stability. This does not eliminate the influence of the charge, because a high charge means a high dissociation and a high hydration. But it makes unnecessary the very doubtful assumption of an electrical repulsion. A repelling force is not necessary to account for the stability. If we assume a "radius of adhesion" within which the attracting force (which certainly does exist) alone is effective, then it is obvious that the micelles will remain dispersed as long as their radius of hydration is greater than the radius of adhesion. Under this condition the Brownian movement is sufficient to account for the dispersion of the colloid. Rapid flocculation will begin when the two radii are equal. This will correspond to a certain "critical potential" which might very well vary, as has been shown, with the nature of the ions, that is, the radius of adhesions might, like the radius of hydration, be a variable factor.

The attracting force

The next question is, what is the nature of the attracting force? Flocculation can hardly be the result of cohesive forces between the molecules of the dispersed phase, because the particles apparently do not come into actual contact. In ageing of a coagulum and in drying, cohesive forces doubtless come into play but flocculation apparently depends upon a looser attachment caused probably by ionic interaction. A "relative displacement by induction

between the two members of the double layer so as to give it an electrical moment," as suggested by Porter and Hedges (25), would account for such an attachment. Several years ago the author offered a somewhat similar explanation. It was assumed that the ions of the outer layer mutually attract the inner layers of two particles thus establishing a linkage between them (15). This would be analogous to the sharing of electrons by atoms forming molecules, as when two H atoms or Cl atoms unite to form a molecule. Our conceptions of atomic structure and micellar structure have much in common. The atom, consisting of a positive nucleus surrounded by the negative electrons, presents an electrical double layer similar to that of the micelle. If the electrically neutral atoms can unite by sharing some of their electrical units, it is only logical to apply the same reasoning to the union of colloidal micelles.

FACTORS GOVERNING THE SIZE OF THE PARTICLES

Size and exchange capacity

Anderson and Mattson (1), who determined ultramicroscopically the particle size in a series of soil colloids, found, as in the case of several other properties, that the dispersibility of the materials varied according to their composition and exchange capacity. Thus the higher the ratio of silica to sesquioxides and the higher the capacity to adsorb and exchange bases, the greater the degree of dispersion. For the two colloids here studied the following figures are presented:

| COLLOID | $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ | EXCHANGE CAPACITY M.EQ./GM. | NUMBER OF PARTICLES PER GM. | AVERAGE RADIUS, $\mu\mu$ |
|---------------|--|-----------------------------------|-----------------------------------|-----------------------------|
| Sharkey | 3.18 | 0.796 | 960×10^{12} | 45.5 |
| Norfolk | 1.63 | 0.207 | 322×10^{12} | 64.5 |

The values represent the dispersion brought about by the addition of NH_3 to the original colloids saturated with various cations and must not be mistaken for "primary particles." For the Na-OH-saturated Sharkey and Norfolk colloids the author estimated, by an indirect method, an average particle radius of 34 and 46 $\mu\mu$ respectively (18).

That a relationship exists between this systematic variation in the size of the particles and the previously discussed colloidal behavior becomes quite evident. Why then are the particles of the Norfolk group of soil colloids larger than those of the group to which the Sharkey belongs? And why are the Na-saturated particles smaller than the K- or NH_4 -saturated?

Before we attempt to answer these questions it is necessary to distinguish between two kinds of particles of colloidal dimensions: (a) Particles formed by the mechanical disintegration of rocks consisting of crystalline rock fragments. Such particles are abundantly present in glacial clays but must exist to some extent in all soils. The size of these articles is the result of a mechanical

accident. (b) Particles formed by the interaction between the hydrous oxides of silicon, aluminum, and iron and the various bases all formed as a result of a chemical weathering of the different minerals. It is this material which possesses the power of base exchange in a high degree and makes up the bulk of the soil colloids here studied. These particles may be looked upon as synthetic aggregates being built up from the various component or constituent parts. The size of the latter particles cannot be accidental but must be the result of an equilibrium between two opposing forces. One of these would expend the free energy by the highest possible degree of aggregation whereas the other, if acting alone, would reduce the free energy content by a higher dispersion. The magnitude of the former would be increased by dispersion, that of the latter by aggregation. The resultant of the two opposing forces must lead to a particle size of such dimensions as to maintain the total free energy at a minimum.

In the first we recognize the force of cohesion represented by the stray fields or residual valences which attract and arrange the molecules in the form of a crystal lattice. As opposed to this force, we must consider the electrokinetic and osmotic forces arising from a dissociation of the complex and resulting in a potential difference, in an osmotic hydration, and probably also in a lowering of the interfacial tension.

Ion density and dispersion

Now it can readily be visualized how these forces operate in the formation of a particle of maximum stability. Let us take a concrete example and consider the Sharkey and Norfolk soil colloids for which data are available. In a Norfolk particle of radius $64.5\mu\mu$ there are about 390,000 exchangeable univalent cations. In a Sharkey particle of the same radius the number of cations would be $.796/.207 \times 390,000 = 1,500,000$. Now if 390,000 cations, at a certain degree of dissociation, are sufficient to overcome the cohesive force, then, is obvious that a Sharkey particle containing nearly four times as many cations would not be stable. The ion density, at the same degree of dissociation, would be too great for stability, thus bringing about a greater dispersion. The Sharkey particle, according to the foregoing data, attains stability at a radius of $45.5\mu\mu$ and a cation content of 505,000. This would, at the same degree of dissociation, still give a considerably higher ion density to the Sharkey than to the Norfolk particle since the surface of a single particle varies as the square of the radius. But according to table 14 (part I), the Norfolk colloid is relatively more dissociated than the Sharkey. It is also obvious that the cohesive force may differ in the two materials. It appears quite evident, however, that the size of the particles is closely related to the number of exchangeable cations just as the osmotic imbibition was found to be so related.

The dispersing action of the dissociated ions may be seen to operate in the following way. The dissociated ions exert an outward pull on the surface layer of ions of unlike sign, thus tending to pull the particle apart. All capillary spaces that might be formed or that are already existing will be filled with liquid

into which the dissociated ions diffuse and establish an osmotic pressure, in excess of that of the outside solution, which will have a tendency to wedge the particles apart. As long as these forces are great enough to overcome the force of cohesion the material will continue to disperse. When once separated the particles are surrounded and protected by a layer of water, the water of osmotic hydration. The dispersing action of the dissociated ions will decrease with the ion density. If the degree of dissociation remained constant the ion density would vary inversely as the surface. For a given quantity of material, the surface varies inversely as the radius, hence the dispersing action of the ions must decrease with a decrease in the radius.

Distinction between aggregation and flocculation

This process of breaking up of coherent aggregates, desaggregation—is independent of the phenomenon of deflocculation. Desaggregation is opposed by the cohesive forces, whereas the forces holding the micelles together in the flocculated condition may be due to an interionic attraction, as has been explained. It is certain that cohesive forces do not here come into play. This can be demonstrated by the following simple experiment. If a piece of bentonite is placed in a NaCl solution of a concentration higher than that required to flocculate, e.g., 0.02 *N*, it will swell considerably but the individual micelles will not diffuse into the supernatant liquid but leave a sharp boundary between the gel and the liquid. If the salt concentration is 0.005 *N* or even 0.01 *N*, the micelles will diffuse into the liquid forming a suspension of decreasing density. In both cases there is a desaggregation but a deflocculation only in the latter. In both cases the cohesive force is overcome by the electrical and osmotic forces of the dissociated ions and the particles are forced apart a distance equal to twice the thickness of the layer of osmotic hydration. At this distance the forces of flocculation come into play in the stronger solution, linking the micelles together. In the weaker solution the micelles are not subject to any mutual attraction and are therefore carried farther apart by the Brownian movement.

Cohesion and aggregation

We have considered the forces leading to a dispersion of larger aggregates into smaller. Let us now consider the conditions which must lead to a condensation or aggregation. Why do the molecules or small units of associated molecules formed in the process of hydrolysis condense into aggregates of such dimensions as we find in the Sharkey and Norfolk soil colloids? To answer this question let us consider a Sharkey and a Norfolk aggregate each so small as to contain only one exchangeable cation. From analytical data (16, 17) the following figures have been calculated, assuming a sp. gr. of 2.65 and a value of 6.06×10^{23} for the Avogadro number:

| COLLOID | RADIUS OF PARTICLE | NUMBER OF EXCHANGEABLE IONS | NUMBER OF MOLECULES OF SiO_2 | NUMBER OF MOLECULES OF $\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ |
|-------------------|--------------------|-----------------------------|---------------------------------------|--|
| | $\mu\mu$ | | | |
| Sharkey | 45.5 | 505,000 | 5,440,000 | 1,710,000 |
| | 0.57 | 1 | 10.8 | 3.4 |
| Norfolk | 64.5 | 390,000 | 12,330,000 | 7,500,000 |
| | 0.88 | 1 | 31.4 | 19.2 |

Two such particles would have a diameter of 1.14 and 1.76 $\mu\mu$, respectively. With a maximum of only one electronic charge, assuming complete dissociation, the particles would be very large as compared to the common ions. Their surface would be about 8 and 19 times as great as that of an ordinary ion, to which we may assign a diameter of 0.4 $\mu\mu$. As ions, they would therefore be very sluggish, showing a slow electrical migration. In this respect they would act like the large complex ions which show only a slow movement in the electric field as compared to the common ions and to the larger colloidal particles which possess a higher ion density.

When this line of reasoning is applied it becomes evident that these particles, although very small, are too large, as ions, to exist alone. The osmotic hydration in the case of a particle dissociating a single ion would, of course, be negligible. The force of molecular cohesion would assume full sway, resulting in a condensation until arrested by an increment of sufficient magnitude in the ion density. Since the number of ions in a particle increases with the cube of the radius, while the surface increases with the square, the increment in ion density would be proportional to the increase in the radius.

It is very improbable that a particle built up in this way would be as large as the Sharkey and Norfolk soil colloid particles. These colloids have been subjected to repeated drying and wetting. Whereas the lyophile colloids, like gelatin and agar, in which the cohesive force is lowered by a high degree of hydration by molecular attraction, may be completely reversible, the reversibility of the suspensoid type of colloids, like those of the soil, is certainly incomplete after the material has once been dried. This was illustrated in table 14, where it was shown that the oven-dried, K-saturated Sharkey colloid would no longer swell. From the great number of undissociated ions present in the soil colloid particles it is evident that if the cohesive force could once be overcome, particles of a much smaller size could be stabilized by an increase in dissociation. An artificial alumino-silicate, having an exchange capacity of the same order of magnitude as that of the natural colloids and which in the Na-saturated condition forms a perfectly clear sol, may be prepared. When dried it is no longer redispersible to the same degree. The condensation-born particle is therefore smaller than the dispersion-born particle. But since there must always be an equilibrium between the two opposing forces, the degree of dissociation in the case of a given material must be greater the smaller the particles.

The relation of the two opposing forces to the radius of the particles is shown graphically in figure 5. The free energy due to molecular attraction, that is to the force of cohesion, increases with the total surface of the dispersed phase and is therefore inversely proportional to the radius of the particles. If the quantity, cohesive force \times surface, is plotted against the radius a curve similar to C and C_1 is obtained. The ion density must vary inversely with the total surface and is therefore directly proportional to the radius as long as the number of ions dissociated remains constant. If the quantity, $\frac{\text{number of ions}}{\text{surface}}$ is

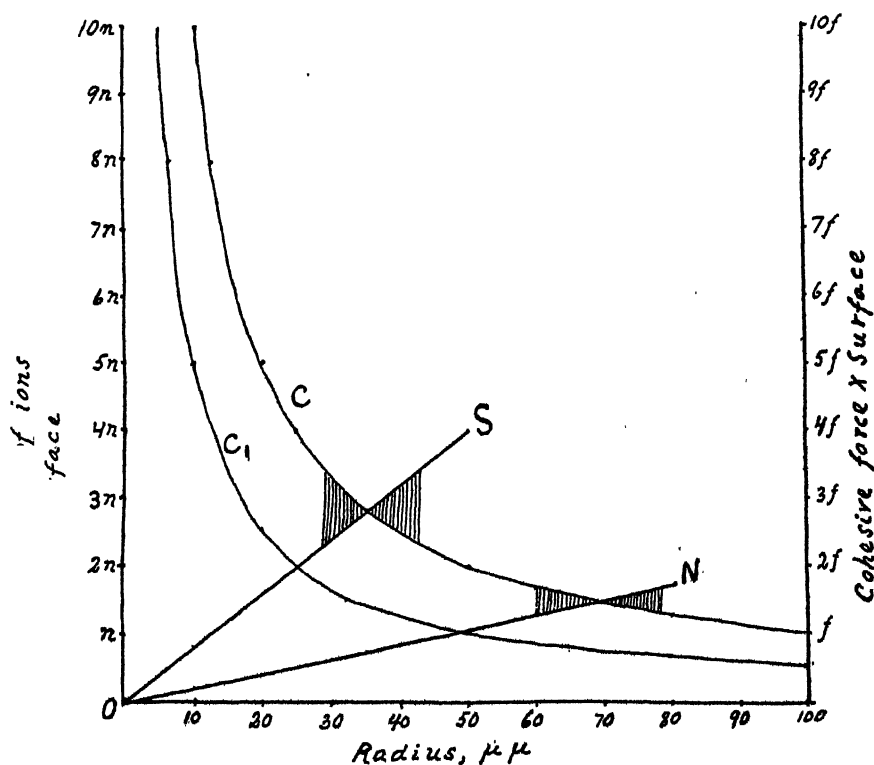


FIG. 5. THE RELATION BETWEEN THE SIZE OF THE PARTICLES, THE SURFACE DENSITY OF IONS, AND THE FORCE OF COHESION

plotted against the radius, curves similar to OS and ON are obtained. The slope of these curves will depend upon the number of ions dissociated. If the degree of dissociation of the Sharkey and Norfolk soil colloids were the same, then the ion density on particles of the same radius would be in the ratio of 0.796:0.207. The slopes of the curves OS and ON are in this ratio. It will be seen that these curves intersect C at points corresponding to radii of the relative values of 36 and 68 respectively. This ratio is a little wider than the ratio

of the radii found for the colloids and would indicate a higher dissociation of the Norfolk. The radius corresponding to the point of intersection represents the radius of the most stable particles. An increase or a decrease in size would make the particles increasingly unstable, as indicated by the shaded sections.

In the case of the same colloid saturated with different bases, the cations of which remain dissociated in various degrees, we obtain the same relationship. Thus let the curve *OS* represent the Na-saturated Sharkey and the curve *ON* the same colloid saturated with a cation only about one-fourth as highly dissociated. The ratio of the radii would again be as represented in the figure.

The curve *C*₁ is plotted to show the influence of a decrease in the force of cohesion, as in the case of the lyophile colloids or that of a lyophile colloid acting as a protecting agent in the formation of a lyophobe colloid. The molecules of a lyophile colloid have a strong attraction for water and a correspondingly weak attraction for one another. Equilibrium between the two forces will therefore be attained at a lower ion density and a correspondingly lower radius, as shown by the points of intersection with curve *C*₁.

Flocculation and charge

We are now in a position, perhaps, to account for the fact that the highly dispersed bentonite shows an increase in the speed of migration in the first stage of flocculation. We have seen how an aggregation of smaller particles into larger, results in an increase in the ion density. This must necessarily result in a corresponding increase in the cataphoretic potential. Although flocculation does not, according to the views here presented, decrease the actual surface of the particles, it will, insofar as the ionic atmospheres overlap one another, cause a crowding and a lateral displacement of the ions. This will increase the mean distance from the inner layer, resulting in an increase in the cataphoretic potential. But since flocculation is conditioned by the presence of a certain quantity of free electrolyte and since all free electrolytes exert a suppressing influence, the aforementioned effect is generally more than balanced by the suppression of the ion activity. Only in the case of a highly dissociated and highly dispersed material like the Na-saturated bentonite does the former effect seem to outstrip the latter. This must be due to the great number of micelles present in each cluster when this suspension is flocculated. Another sample of bentonite from a South Dakota deposit, saturated chiefly with Ca and Mg and only very slightly dispersible even after saturation with Na, did not show the above anomaly (16).

In closing, another flocculation anomaly, unexplainable on the basis of the generally accepted theory, will be given. The Aragon soil colloid which was found to be isoelectric in old, CO₂-contaminated water did not flocculate in this condition, only a slow sedimentation, due to the large size of the particles, being observed. The addition of 2 milliequivalents Ca(OH)₂ per liter resulted in rapid flocculation although the particles migrated now toward the anode with a speed of 0.8μ per second. In the isoelectric condition the particles can have no

ion atmosphere. The addition of $\text{Ca}(\text{OH})_2$ resulted in a negative charge, indicating a cation atmosphere. Thus it appears very much as if flocculation, which must be looked upon as a clustering of the micelles and not as a molecular union of the particles, is conditioned by the existence of an ion atmosphere. This would be in harmony with the theory of ion linkage, according to which the particles are linked together by the ions of opposite charge. Where there is no ion atmosphere there is no connecting links and no flocculation. Aggregation by molecular attraction is then alone possible. But an ion atmosphere is alone not sufficient to attract and link the micelles together. Some stability factor such as hydration and possibly also molecular orientation must be broken down. This is accomplished by the free electrolyte, the presence of which appears to be essential to the process of flocculation. Whereas the forces of molecular aggregation and desaggregation, which determine the particle size, operate under all conditions, flocculation is the result of a more or less abrupt change in the stability of the micelles, brought about at a critical concentration of free electrolytes. Thus we find all sizes of particles in stable conditions depending on the nature of the material and the ions in combination.

SUMMARY

Flocculation and cataphoresis in solutions of various electrolytes and mixtures of electrolytes have been studied in the case of bentonite and soil colloids having different silica/sesquioxide ratios and different exchange capacities.

The charge and stability of the colloid depend upon the degree of dissociation of the exchangeable ions, which varies with their valence, hydration, potential, etc.

The theory which connects the charge and the stability with an adsorption of the ions of the added electrolyte is not supported. Thus the ions of the same sign as that of the colloid influence the stability according to the valence rule as established for the suppression of the swelling, although the ions are negatively adsorbed. The same must be true of the ions of opposite sign of charge, but here the valence effect is greatly exaggerated because of the formation of a new complex by exchange with each change of ion. The ions of opposite sign always enter into an exchange reaction with the ions present in the complex. If the ingoing ion is more highly dissociated by the complex than the ion originally present, then the charge, the osmotic hydration, and the dispersion tend to increase; if less dissociated, these values will all decrease and the sensitivity to electrolytes will increase. The free electrolyte exerts only a suppressing effect, and this in accordance with the valence and concentration of the ions. But the suppression of the charge and of the stability by free electrolytes is not, in the case of the common ions, due to an adsorption of the ions of opposite sign of charge except insofar as an exchange takes place; nor is it due to a suppression of the dissociation except insofar as the ingoing ion is less dissociated by the complex. On the contrary, the addition of electrolytes always increases the dissociation of the colloidal complex. This is identical with the salt effect on

weak electrolytes and is explained by a decrease in the activity of the ions according to the theory of interionic attraction. The abnormally large observed increase in dissociation is accounted for by the highly multivalent character of the particles. A linear relation between the dissociation and the square root of the ionic strength was brought out. An examination of the data supplied by Loeb reveals a similar relationship in the case of the proteins.

The quantity and quality of the exchangeable ions determine, through their various degrees of dissociation, the colloidal behaviors such as charge, osmotic hydration sensitivity to electrolytes, and dispersibility, that is, the size of the particles as determined by molecular aggregation.

The particle size is seen as the result of a balance between the force of cohesion on the one side and the force of dispersion, as determined by the ion density, on the other. This will result in a size of maximum stability for each colloid, depending on the nature and quantity of exchangeable ions. The relation between the observed size of particles in different colloids and the exchange capacity is brought out in a graph in which the ionic density and cohesive energy is plotted against the radius.

A distinction is made between aggregation and flocculation. Aggregation (and this also applies to disaggregation), which determines the size of the particles, is a continuous process which operates under all conditions. Flocculation is a discontinuous process in which the micelles (not the particles) are linked together, apparently by an electrostatic attraction. It seems to depend upon the existence of an ion atmosphere but takes place only in the presence of free electrolyte, the amount of which depends more on the degree of hydration than on the magnitude of the charge. There is no one critical potential at which soil colloids flocculate. Flocculation is often observed at a higher cataphoretic potential than that of the original stable suspension.

The osmotic hydration is the most potent stability factor. Thus the concentration required to flocculate a Na-saturated colloid having a high exchange capacity, which means a high osmotic hydration, is very much higher than in the case of a Na-saturated colloid with a low exchange capacity, although the initial charge may be the same. This difference in stability vanishes if the colloids are dehydrated with alcohol, in which condition they become extremely sensitive to electrolytes.

Although dispersion and particle size are held to be closely related to the charge, it is very doubtful whether the stability of the micelles depends upon a mutual repulsion. The osmotic hydration of the micelles is sufficient to account for the stability of a suspension. Since this hydration depends upon the number of dissociated ions, the connection between a high charge and the stability of a suspension, which is often observed but which is not general, is seen as an indirect effect.

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EXTRACTION OF ADSORBED CATIONS FROM SOIL BY ELECTRODIALYSIS

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Cations are extracted differentially from soil by electrodialysis. Mattson (6) found, by means of fractional electrodialysis, that calcium was removed with relatively greater ease from the colloidal material extracted from a soil than was magnesium, iron, or aluminum. In like manner, but using a different type of electrical cell, the writer (8) observed this same cationic relationship to hold with respect to a clay soil. In each of these experiments the material which was subjected to electrodialysis contained all of the cations which were characteristic of it. Of these calcium predominated.

The experiments of the present investigation differ from those just cited in that they are concerned with the ease or rapidity with which certain cations appear in the diffusates of electrodialyzed soil when it is saturated with respect to a single cation or a particular combination of cations.

DESCRIPTION OF SOIL

A soil designated as Vergennes clay was employed in the investigation. It is a productive soil derived from the decomposition of glacial-lake clay.¹ It contains approximately 12 mgm. equivalents of exchangeable cations to 100 gm. of air-dry soil as determined by electrodialysis. It has a reaction of pH 5.4 and is classified as a soil of good fertility. In preparation for analysis the soil was air-dried and passed through a-1 mm. sieve. Stones larger than 1 mm. were discarded.

METHODS EMPLOYED

Ten grams of soil were electrodialyzed by the use of a two-compartment-Pyrex cell (8) similar in design to the one devised by Bradfield (1). Electrodialysis was conducted for 8 hours under the influence of a direct current of 110 volts, which was regulated by means of a sliding-contact rheostat. During this period about one liter of diffusate was obtained. The amperage varied during the process of dialysis; it was seldom higher than 100 milliamperes; at the close of the process it was occasionally as low as 5 milliamperes.

¹ The soil contains 4.3 per cent, 1.4 per cent, and 3.1 per cent respectively of calcium, magnesium, and potassium.

After being subjected to electrodialysis, the soil was freed of soluble anions by means of distilled water and suction while in the alundum thimble in which electrodialysis occurred. At this stage the alumino-silicic complex of the soil was more or less saturated with hydrogen containing few or no other exchangeable cations, and the reaction of the soil approached pH 4.0. The soil was then air dried and transferred to a small beaker. Any soil adhering to the walls of the thimble was loosened and washed into the beaker with a part of the solution containing the cation or cations with which the soil was to be treated. In most instances this was a normal solution of a salt of acetic acid or a combination of several salts of acetic acid in molecular equivalent quantities. One hundred cubic centimeters of solution was added to the soil and the mixture frequently stirred. The soil was allowed to settle for 24 hours and the supernatant liquid was then withdrawn with a pipette. This procedure was repeated successively with two fresh portions of solution, after which the soil was washed several times with distilled water by decantation and transferred to an alundum thimble. The washing was continued, suction being used, until the filtrate gave no test for the ions with which the soil had been treated. When air dry, the soil was brought into suspension with a small quantity of distilled water and electrodialyzed in the manner already described to remove the particular cation or cations which it had adsorbed.²

The total quantity of cations in the diffusates was determined by titration with suitable reagents (8). Individual cations were ascertained by standard methods of chemical analysis. Hydrogen-ion concentrations were determined potentiometrically, a quinhydrone electrode being used.

CATIONS EXTRACTED BY ELECTRODIALYSIS FROM SOIL SATURATED WITH HYDROGEN BEFORE TREATMENT

The cations present in the diffusates of the electrodialyzed soils, after being subjected to the several treatments outlined in the foregoing, are recorded in table 1. Normal solutions of potassium, calcium, and magnesium acetates were employed. The solution of aluminum acetate was approximately 0.6 *N*, its being the limit of solubility in distilled water of the salt at hand. As indicated in the table, electrodialysis was conducted fractionally. The first fraction (A) represents the cations which were extracted from the soil during a period of 2 hours; the second (B) and the third fractions (C) each represent the quantity extracted during a period of 3 hours. The total quantity of cations in each fractional diffusate, determined by titration and expressed in terms of NaOH, may be compared with its content of the cation with which the soil was treated. Such a comparison shows that virtually the only cation present in the diffusates was the one which was introduced into the soil.

Practically equivalent quantities of cations were extracted from the soils treated with potassium or calcium acetate. But potassium was removed from

² The word "adsorption," as used in this paper, is not intended to distinguish between physical and chemical forces.

the soil with greater rapidity than was calcium. This may be seen by comparing the quantities of the cations present in the diffusates. Five hours were required to extract as much calcium from the soil as was extracted in two hours with respect to potassium. Magnesium was removed with comparative difficulty from the soil treated with mangesium acetate. The quantity extracted in 8 hours was approximately 50 per cent of the quantity of potassium or calcium removed during a period of equal length. Although the amount of magnesium was smaller in each successive fraction, its rate of decrease was exceedingly small as compared with that of potassium or calcium.

TABLE 1
Cations extracted from soil by fractional electrodialysis
(Soil electrodialyzed before treatment)

| TREATMENT | FRACTION | MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL | |
|------------------------|----------|--|--------------------------------------|
| | | Cation of treat- ment | Total cations in terms of NaOH |
| Potassium acetate..... | A | 17.8 | 18.6 |
| | B | 1.5 | 2.4 |
| | C | 0.2 | 0.4 |
| | Total | 19.5 | 21.4 |
| Calcium acetate..... | A | 12.1 | 12.5 |
| | B | 5.4 | 6.2 |
| | C | 1.1 | 1.4 |
| | Total | 18.6 | 20.1 |
| Magnesium acetate..... | A | 3.8 | 3.7 |
| | B | 2.8 | 3.0 |
| | C | 2.5 | 2.1 |
| | Total | 9.1 | 8.8 |
| Aluminum acetate..... | A | 0.2 | 0.3 |
| | B | 0.1 | 0.1 |
| | C | 0.1 | 0.1 |
| | Total | 0.4 | 0.5 |

Very little aluminum was found in the diffusates of the soil treated with aluminum acetate. Less than 1 mgm. equivalent was removed to the cathode chamber during 8 hours of electrodialysis. The limited quantity of magnesium and the exceedingly small quantity of aluminum extracted from the soil, as compared with the quantities of potassium and calcium extracted, raised the question as to the actual amounts of magnesium and aluminum adsorbed by the soil. If these latter cations were adsorbed to the same degree as were potassium and calcium, it is obvious that their behavior with respect to elec-

trodialysis differed fundamentally from that of the other two cations. This phase of the subject will be discussed presently.

With aluminum omitted from the discussion for the present, it may be said that the ease with which the following cations were removed by electrodialysis from the soil of the investigation, when only one of them was presented for adsorption, was in the descending order $K > Ca > Mg$. When they were adsorbed in the presence of one another the same order prevailed. This is shown in table 2. The data there recorded were obtained as a result of treating the soil with a normal solution of potassium, calcium, and magnesium acetates in molecular equivalent quantities. The method of treating the soil was precisely that which was employed when the soil was treated with only one of these cations. Potassium was removed with the greatest rapidity, followed by calcium and magnesium in the order named. A direct relationship is seen to exist between the ease of cationic extraction and the quantity of cations removed. Potassium, which was removed with the greatest rapidity, was also

TABLE 2

Cations extracted by fractional dialysis from soil treated with potassium, calcium, and magnesium in molecular equivalent quantities
(Soil electrodialyzed before treatment)

| FRACTION | MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL | | | | |
|------------|---|-----|-----|-------|--------------------------------|
| | K | Ca | Mg | Total | Total cations in terms of NaOH |
| A | 8.1 | 3.3 | 0.8 | 12.2 | 11.8 |
| B | 1.4 | 3.6 | 1.8 | 6.8 | 6.0 |
| C | 0.5 | 0.4 | 1.7 | 2.6 | 1.8 |
| Total..... | 10.0 | 7.3 | 4.3 | 21.6 | 19.6 |

removed in largest amount. The converse was true for magnesium. The sum of the quantities of the three cations extracted from the soil in 8 hours was in stoichiometric agreement with the quantity of potassium or calcium removed during an equal period of time when the soil was treated with one or the other of these cations. This relationship would imply that most of the magnesium which was adsorbed in the presence of potassium and calcium was extracted in 8 hours. This was probably the case, for it would seem from the data presented that the adsorption of magnesium, under the conditions of the experiment, was relatively small. In that event, any magnesium that may have remained in the alumino-silicic complex of the soil after 8 hours of electrodialysis was not in sufficient quantity to affect materially the total amount of cations recovered during that time.

It is not to be assumed that complete saturation of the soil occurred with respect to any one of the treatments. It seems probable, however, in the light of the results shown in tables 1 and 2, that the soil was near the point of cationic

saturation after treatment with potassium or calcium acetate or a combination of these with magnesium acetate.

Cations extracted by electrodialysis from soil saturated with calcium before treatment

In the experiments just reviewed the aluminosilicic complex of the soil was saturated with hydrogen before the soil was treated with the cations of the acetate solutions. When the complex was in that condition, as has been shown, the total recovery of the added cations from the soil by means of electrodialysis was decidedly different. The difference in the recovery of potassium and calcium on the one hand, and magnesium and aluminum on the other, suggested that magnesium may react to replace only a fraction of the exchangeable hydrogen in the aluminosilicic complex of the soil and that aluminum may not cause the replacement of any of it. This contingency led to the following experiment which was designed to determine whether more of the

TABLE 3

Calcium removed from soils treated with calcium acetate by potassium, magnesium and aluminum acetates and the extraction of these latter cations by electrodialysis
(Soils electrodialyzed before treatment with calcium acetate [pH 7.6])

| FINAL TREATMENT OF SOIL | MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL | | |
|--------------------------------------|---|---------------------------|---|
| | Ca removed by K, Mg, or Al acetate | K, Mg, or Al in diffusate | Total cations in diffusate in terms of NaOH |
| Potassium acetate (pH 7.7) | 18.1 | 18.9 | 20.3 |
| Magnesium acetate (pH 7.5) | 18.2 | 7.1 | 9.0 |
| Aluminum acetate (pH 3.5) | 17.8 | | 0.6 |

magnesium and aluminum added to the soil as acetates could be recovered by electrodialysis if the replaceable hydrogen of the soil was first replaced with calcium. That comparisons might be made between the action of magnesium and aluminum with that of potassium, tests using potassium acetate were conducted concurrently.

To this end soils were electrodialyzed and treated with calcium acetate. If a soil with its aluminosilicic complex saturated with calcium is treated with potassium, magnesium, or aluminum acetate and the supernatant liquid and wash water resulting from each of the treatments are analyzed for calcium, it should be possible to ascertain the extent to which each of these three cations will bring about the release of the adsorbed calcium. If the soil resulting from each of the three treatments is electrodialyzed, it should be possible to compare the degree of extractability of potassium, magnesium, and aluminum with the quantity of calcium released by each of the treatments.

The results which were obtained in following these two procedures are re-

corded in table 3. Each cation is seen to have liberated to the supernatant liquid approximately equal quantities of calcium. The values referred to are slightly less than the quantity of calcium that was obtained by electro dialyzing the soil when saturated with calcium acetate, as is shown in table 1. Nevertheless, the differences are within the limits of probability and it appears safe to conclude that each of the three cationic treatments released from the soil an equal quantity of adsorbed calcium. But the quantities of cations recovered from the residual soil by electro dialysis do not indicate that, with the replacement of the adsorbed calcium, equal quantities of potassium, magnesium, and aluminum entered the aluminosilicic complex of the soil. Potassium, no doubt, did enter the adsorbing complex stoichiometrically in replacing calcium. But the results of table 3 do not show this relationship to obtain with respect to magnesium or aluminum. Aluminum was not determined in the diffusate of the soil treated with aluminum acetate. Its determination did not appear necessary because of the small total cationic content of the diffusate as determined by titration. This value is shown in terms of NaOH. It is not clear from the data presented whether magnesium and aluminum entered the soil complex as exchangeable ions and were not extractable by electro dialysis to the same extent as was potassium, or whether they were precipitated within the soil mass, bringing about the liberation of calcium by means of secondary reactions. Kelley and Brown (4) have expressed the view that cationic replacement in soil resulting from the addition of aluminum chloride is induced by the presence of hydrogen ions resulting from the hydrolysis of the aluminum salt and not by the aluminum ions directly.

Adsorption of calcium by electro dialyzed soils after treatment with magnesium or aluminum

In an attempt to determine the degree to which magnesium and aluminum entered the adsorbing complex of the electro dialyzed soil, it was treated first with magnesium or aluminum acetate and later with calcium acetate. It appeared to the writer that by analyzing the supernatant liquids resulting from the calcium-acetate treatments for magnesium and aluminum respectively, and by electro dialyzing the residual soils, some knowledge of the quantities of these cations which entered the adsorbing complex of the soil might be ascertained. The intent of this latter statement is apparent from table 4.

It will be seen that approximately 20 mgm. equivalents of magnesium was extracted from the soil when it was treated with calcium acetate. In the liberation of the magnesium an equal quantity of calcium was adsorbed by the soil. This is shown by the quantity of calcium which was present in the diffusate of the soil that had been treated with magnesium acetate when it was electro dialyzed. No other cation was present in the diffusate in appreciable amount, for its total cationic content was practically that of its calcium content. The quantity of magnesium shown to be liberated from the soil is equivalent to the quantity of calcium that was found to be adsorbed by the soil in the earlier ex-

periments. The data in table 4 which refer to magnesium, together with certain of those in tables 1 and 3, appear to support the conclusion that the soil of the investigation, after being subjected to electrodialysis, adsorbed equal quantities of potassium, calcium, and magnesium as acetates but that the latter cation was more difficult of extraction by electrodialysis than was either potassium or calcium.

Only a trace of aluminum was obtained from the soil previously treated with aluminum acetate when it was brought in contact with calcium acetate. Still, it is not surprising that aluminum did not appear in the supernatant liquid resulting from the calcium-acetate treatment. If aluminum had been released from the soil, it would undoubtedly have been precipitated as aluminum hydroxide in the presence of calcium acetate of reaction pH 7.6. If aluminum had entered the aluminosilicic complex of the electrodialyzed soil by replacing its exchangeable hydrogen ions and was not in turn replaceable by calcium, it does not seem that the soil should have adsorbed calcium. But it did ad-

TABLE 4

Removal of magnesium and aluminum from soils by calcium acetate and the extraction of calcium from the residual soils by electrodialysis

(Soils electrodialyzed before treatment with magnesium or aluminum acetate)

| TREATMENT OF SOIL | MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL | | |
|----------------------------------|---|-----------------|---|
| | Mg or Al removed by Ca acetate | Ca in diffusate | Total cations in diffusate in terms of NaOH |
| Magnesium acetate (pH 7.5) | 20.2 | 19.8 | 20.2 |
| Aluminum acetate (pH 3.5) | Trace | 11.1 | 11.3 |

sorb calcium, for 11.1 mgm. equivalents was extracted from the soil by electrodialysis. If the soil had not been previously treated with aluminum acetate, approximately 20 mgm. equivalents of calcium would have been extracted. The effect of the aluminum-acetate treatment was to reduce considerably the adsorptive power of the soil for calcium or to reduce the rapidity of its removal by electrodialysis. This has some relation to the findings of Magistad (5), who has reported that the addition of aluminum to an alkaline soil with which he worked resulted in destroying its capacity to adsorb barium.

Adsorption of aluminum by soil saturated with hydrogen or calcium

The action of aluminum acetate in reducing the adsorptive power of the electrodialyzed soil for calcium or in retarding its extraction by electrodialysis was an indication that the soil possessed the property of adsorbing aluminum from aluminum acetate when its aluminosilicic complex was saturated with hydrogen and when its hydrogen-ion concentration was approximately 4.0. To test this possibility, an experiment was designed in which 10 gm. of

electrodialyzed soil was treated with 50 cc. of 0.3 *N* aluminum acetate. After the mixture had stood in a closed vessel for 24 hours, aluminum was determined in a part of the clear supernatant liquid. A similar test was conducted with soil which had been electrodialyzed and subsequently saturated with calcium by means of calcium acetate. The results of the experiment are to be seen in table 5.

About 15 mgm. equivalents of aluminum were adsorbed from the solution by the electrodialyzed soil and about 24 mgm. equivalents by the electrodialyzed soil which had been saturated with calcium acetate. Although the calcium acetate saturated soil contained approximately 20 mgm. equivalents of calcium, only 9.8 mgm. equivalents were extracted by treatment with aluminum acetate. The quantities of calcium, magnesium, and potassium which were extracted from each of the soils will not account for the quantity of aluminum that was removed, and it is not likely that other cations were extracted from the soils in more than minute quantities.

TABLE 5
Removal of aluminum from aluminum acetate by soil saturated with hydrogen or calcium
(Soil electrodialyzed before treatment)

| STATE OF SOIL | MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL | | | | pH OF SOLUTION BEFORE TREATMENT | pH OF SOLUTION AFTER TREATMENT |
|--------------------------|--|-------------------------------|-------------------------------|------------------------------|--|---|
| | Al removed from solution | Ca removed from soil | Mg removed from soil | K removed from soil | | |
| Hydrogen saturated | 14.9 | Trace | 0.9 | 1.3 | 3.78 | 3.93 |
| Calcium saturated | 24.2 | 9.8 | Trace | 0.5 | 3.78 | 3.85 |

The process by which aluminum was adsorbed by the soils is not entirely clear. It might be assumed in the case of the acid soil that aluminum replaced hydrogen ions. However, this is not reflected in the hydrogen-ion concentration of the aluminum-acetate solution after it had been in contact with the acid soil. Perhaps it is of some significance that the aluminum adsorbed by the soil treated with calcium acetate, whose reaction was pH 7.4, was essentially equivalent to that adsorbed by the acid soil plus the calcium liberated from the alkaline soil. Thus, it appears that the alkaline soil may have adsorbed as much aluminum as did the acid soil before the calcium was liberated. If the alkaline soil had been treated with a sufficient quantity of aluminum acetate, all of its exchangeable calcium would, no doubt, have been removed.

DISCUSSION

Under the conditions of these experiments and when present individually, potassium, calcium, and magnesium were adsorbed stoichiometrically by electrodialyzed soil and also by soil that had been electrodialyzed and later

made alkaline by treatment with calcium or magnesium acetate. But the manner of their extraction by means of electrodialysis was distinctly different. Although potassium and calcium were removed in equivalent quantities during a period of 8 hours, potassium was removed with greater rapidity than was calcium. The recovery of magnesium was exceedingly slow—less than one half of the quantity known to be adsorbed by the soil was extracted by electrodialysis in 8 hours.¶

The magnitude of the adsorption of the three cations when added to electrodialyzed soil in molecular equivalent quantities was in the same order as the ease with which they were extracted from the soil by electrodialysis. Thus, more potassium was adsorbed than calcium, and more calcium than magnesium. Although these cations were adsorbed in different quantities, the sum of the quantities so removed was equivalent to that which occurred when each of them was adsorbed in the absence of the other two. Gedroiz (3) found the energy with which barium was replaced from soil by calcium, magnesium, and potassium to decrease in the order named. The present experiment shows potassium to have a greater replacing value than either calcium or magnesium when co-existent in molecular equivalent quantities. No doubt the difference in the order of replacement referred to is attributable to the widely varying conditions under which the two experiments were conducted.

Only an inappreciable quantity of aluminum could be extracted by electrodialysis from electrodialyzed soil that had been treated with aluminum acetate. Nor could larger quantities be extracted in this manner from electrodialyzed soil that had been made alkaline by means of calcium acetate before treatment with aluminum acetate. Although little or no aluminum was extracted from the soil, the soil is known to possess the property of adsorbing aluminum from aluminum acetate when in the two states previously mentioned; that is, when its aluminosilicic complex is saturated with hydrogen or with calcium. When the soil which contained replaceable calcium was treated with aluminum acetate, calcium was liberated. This replacement may have been induced by hydrogen ions rather than by aluminum ions, according to the views of Kelley and Brown (4). But in the case of the acid soil, whose exchangeable cations had been removed by electrodialysis, aluminum may have been adsorbed, not through the precipitation of aluminum as aluminum hydroxide, but in such a manner as to become a part of the colloidal complex of the soil. If this occurred, the effect of the aluminum acetate in reducing the adsorptive capacity of the electrodialyzed soil for calcium might be explained on the assumption that aluminum is held very tenaciously in the colloidal complex of the soil and is removed with difficulty by means of calcium acetate.

Unpublished data obtained in this laboratory have shown that aluminum chloride and aluminum acetate of the same normality react differently with the electrodialyzed soil of the investigation. Aluminum was not adsorbed by the soil when treated with aluminum chloride nor did the treatment interfere more than slightly, if at all, with the subsequent adsorption of calcium by the

soil. Mattson (7) has suggested that soil material does not adsorb aluminum from aluminum chloride because of the acid reaction of the salt or because it renders the soil material electropositive, in which condition it loses its ability to adsorb cations. The failure of the electrodialed soil to adsorb aluminum from aluminum chloride was probably due to the acidity of the aluminum chloride induced by the strong chlorine ion. If the treatment had caused the soil to become electropositive it would not have adsorbed calcium, which it accomplished with comparative ease.

Although the process by which aluminum is adsorbed by soils is a matter of conjecture, it does not appear improbable that, under certain conditions, it enters into combination with the colloidal complex of the soil.

It has been suggested by Cooper et al. (2), that a relationship exists between the standard electrode potentials of the elements and the order of their removal from soil by electro dialysis. The relative ease with which the adsorbed cations of the present investigation were recovered by electro dialysis conforms to this suggestion.

SUMMARY

Potassium, calcium, magnesium, and aluminum acetates were added to electrodialed soil and also to soil that had been electrodialed and subsequently made alkaline by treatment with calcium or magnesium acetate. The rapidity and the comparative extent of their recovery from the soils were ascertained by means of electro dialysis.

The degree to which the cations were extracted from the soil when in either of the states mentioned, was essentially the same. When each cation was adsorbed at the exclusion of the others, potassium and calcium were recovered by electro dialysis in equivalent quantities and to a considerably greater degree than was magnesium. This occurred in spite of the fact that the soil adsorbed as much magnesium as it did potassium or calcium.

Aluminum was adsorbed by the soil when its aluminosilicic complex was saturated with hydrogen or with calcium, but only minute quantities of it could be extracted by electro dialysis. The manner in which it was adsorbed is not clear, but it appears that under certain conditions it might become a part of the aluminosilicic complex of the soil.

Potassium, calcium, and magnesium added to electrodialed soil in molecular equivalent quantities were extracted, collectively, to the extent that each of them was extracted when adsorbed individually. When they were adsorbed in the presence of one another more potassium was adsorbed than calcium, and more calcium than magnesium. The ease of their recovery, by electro dialysis, was in the same order as the magnitude of their adsorption.

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THE VALUE OF RAW SEWAGE SLUDGE AS FERTILIZER¹

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The disposal of sewage sludge is, in most communities, a problem of vital importance. With the development of the activated sludge system, the use of this type of sludge as fertilizer is coming into favor. There are also a number of disposal plants which sell sludge, as fertilizer, from units in which the sewage solids are decomposed, the decomposition usually amounting to 40-50 per cent of the volatile matter. The sludge, which is sold at a low price, contains about one-half the nitrogen of fresh sewage solids. Little, however, seems to have been done in studying the actual fertilizer value of raw, fresh sludge, dried and ground.

The Atlantic City Sewerage Company, being situated in a community built on white sea sand which is practically devoid of organic matter, felt that there were some interesting economic possibilities in the use of the raw sludge as a soil builder. Hence the study reported in this paper was undertaken.

THE MATERIAL

At the Atlantic City disposal plant the fresh settled solids are pumped daily to sand filter beds. Samples were taken at different times from these beds, allowed to thoroughly air dry, and were then finely ground. The material, after being ground, was very light and fluffy.

These samples were analysed by the official methods of the Association of Official Agricultural Chemists. All analyses were made in duplicate or triplicate. The results are shown in table 1. It is seen that phosphoric acid and potash are present in almost negligible quantities. The carbon-nitrogen ratio is 14.3, which, as is shown in pot series 3, is too wide for optimum plant growth. Chlorides and sulfates are present only in traces.

The total nitrogen content of raw sewage sludge, as shown by analyses of the department of sewage disposal, is usually between 4.5 and 5 per cent. The

¹ The work herein reported was carried on for the Atlantic City Sewerage Co., Atlantic City, N. J., Mr. C. G. Wigley, Engineer; and it is with their kind permission that this paper is published.

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low nitrogen content of the Atlantic City fresh solids might be due to (a) a high ash content probably caused by infiltration carrying sand, or (b) the type of treatment which allows a good deal of the organic constituents to go through the tanks.

POT EXPERIMENTS

Because the crops growing on an experimental plot at the Atlantic City Sewerage Company plant were destroyed by a heavy northeast storm, this paper deals only with the results obtained in the greenhouse of the department of soil chemistry and bacteriology, New Jersey Agricultural Experiment Station.

TABLE 1
Analysis of dried, fresh sewage sludge

| | NUMBER OF ANALYSES | AVERAGE |
|-------------------------------|-----------------------|-----------------|
| | | <i>per cent</i> |
| Moisture..... | 4 | 10.22 |
| Ash..... | 4 | 37.45 |
| Total carbon..... | 2 | 28.10 |
| Total nitrogen..... | 5 | 1.96 |
| Ammonia nitrogen..... | 2 | 0.12 |
| "Available" nitrogen..... | 2 | 0.76 |
| Total phosphoric acid..... | 2 | 0.62 |
| Total potash..... | 1 | 0.13 |
| Iron and aluminum oxides..... | 1 | 1.19 |
| pH of water extract..... | 3 | 5.66 |

Series 1; Part 1: Beets

Beets were grown in Sassafras sand in glazed earthenware pots. The various fertilizers were mixed with 10 pounds of soil in each case, and in such quantities as to add 225 mgm. of nitrogen to each pot. The plants were thinned to five to each pot two weeks after planting, and harvested two months after planting. The results are given in table 2, the green weights being referred to a base of the checks equal to 100. The results are the averages of duplicate treatments. The fertilizer mixtures were made up as shown in table 3.

Series 1; Part 2: Barley

After the beets were harvested, the pots were allowed to stand fallow for three weeks. Barley was then planted and was harvested two months later. The results are given in table 2 under "Barley," the figures being relative green weights of tops. It should be pointed out that although the checks are rated at 100 in the cases of the beets, barley, and combined crops, and the rest of the weights are referred to those bases, the actual weights in all three cases were, of course, widely different.

The principal facts brought out in table 2 are: first, the mineral fertilizer increased the beet growth greatly over that of the check, but was largely used up by this crop, and the residual crop, barley, made comparatively little better growth than the check; second, the sludge had practically no beneficial action when used alone on either beets or barley, and in the case of the barley where no lime was used, actually decreased the yield below that of the check; third, it appears that phosphoric acid is a limiting factor, in the case of beets at least, since mixture IV containing no phosphate gave very poor growth.

TABLE 2
Beets and barley

| POT NUMBER | TREATMENT | RELATIVE GROWTH | | |
|------------|---------------------------|-----------------|--------|----------------|
| | | Beets | Barley | Combined crops |
| | <i>gm.</i> | | | |
| 1 | Nothing (check) | 100 | 100 | 100 |
| 2 | 4.5 5-8-5 mineral mixture | 4,380 | 127 | 698 |
| 3 | 4.5 5-8-5 mineral mixture | | ... | ... |
| | 4.3 lime | 5,340 | 108 | 810 |
| 4 | 15.0 dried sludge | 110 | 63 | 70 |
| 5 | 15.0 dried sludge | | ... | ... |
| | 4.3 lime | 200 | 111 | 123 |
| 6 | 7.5 sludge mixture I | 850 | 168 | 259 |
| 7 | 6.5 sludge mixture II | 1,030 | 123 | 245 |
| 8 | 5.0 sludge mixture III | 540 | 141 | 195 |
| 9 | 5.5 sludge mixture IV | 30 | 64 | 59 |

TABLE 3
Fertilizer mixtures
(Parts by weight)

| MIXTURE | ANALYSIS | NITRATE OF SODA | SUPER-PHOSPHATE | MURIATE OF POTASH | DRIED SLUDGE |
|-----------------|----------|----------------------------|-----------------|-------------------|--------------|
| Mineral..... | 5-8-5 | 3.0 | 5 | 1.0 | .. |
| Sludge I..... | 3-5-2 | 3.0 | 6 | 1.0 | 10 |
| Sludge II..... | 3-8-4 | 4.5 | 10 | 1.5 | 4 |
| Sludge III..... | 4-7-4 | (5 nitro-phoska; 5 gypsum) | | | 10 |
| Sludge IV..... | 4-0-3 | 3.8 | .. | 1.2 | 10 |

Series 2: Beets

In series 2 the same kind of soil was used as before, and beets were again the crop grown. Ten pounds of soil to each pot was used. Table 4 gives the treatments and the results, relative green weights referred to the check as 100.

The treatment in pot 2 was at the rate of 10 tons of dried sludge to the acre. The results of this series indicate that a sufficiently heavy application of sludge is about as efficient as sludge supplemented by phosphate or potash. Considerable benefit was derived from the application of nitrogen.

Series 3: Beets

Series 3 was planned to determine the effect of narrowing the carbon-nitrogen ratio of the dried sludge by the addition of available nitrogen in the form of nitrate of soda. The same kind of soil was used as in series 1 and 2, and beets were again planted. Superphosphate and muriate of potash were added to all pots alike, including the check. Four mixtures were prepared having carbon-nitrogen ratios varying from 13.3 to 3.3. White quartz sand was added to give a uniform proportion of sludge to minerals in all of the

TABLE 4

Beets

| POT NUMBER | TREATMENT | RELATIVE GROWTH |
|------------|--------------------------------------|-----------------|
| | <i>gm.</i> | |
| 1 | Nothing (check) | 100 |
| 2 | 45 dried sludge | 250 |
| 3 | 15 dried sludge, 2 nitrate of soda | 340 |
| 4 | 15 dried sludge, 5 superphosphate | 220 |
| 5 | 15 dried sludge, 2 muriate of potash | 230 |

TABLE 5

Beets

| POT NUMBER | TREATMENT | N:C | N | RELATIVE GROWTH |
|------------|---|--------------------------|-------------------------|-------------------|
| | | | <i>mgm.</i> | |
| 1 | 15 gm. 15 parts sludge 5 parts sand | 1:13.3 | 208.5 | 100 ... |
| 2 | 15 gm. 15 parts sludge 1 part nitrate 4 parts sand | 1: 8.6 | 321.0 | 210 |
| 3 | 15 gm. 15 parts sludge 3 parts nitrate 2 parts sand | 1: 5.0 | 546.0 | 250 |
| 4 | 15 gm. 15 parts sludge 5 parts nitrate | 1: 3.3 | 846.0 | 230 ... |

mixtures. The treatments and results, relative green weights on the basis of the check as equal to 100, are shown in table 5.

The results indicate that a narrowing of the carbon-nitrogen ratio to about 8 is desirable, but uneconomical below this. This would explain the stimulating effect of the added nitrogen in series 2, since there the ratio was reduced to 1:8.

Series 4; Part 1: Grass

A quantity of sea sand was obtained from Atlantic City for series 4, and a commercial lawn grass seed was planted in it. After an initial vigorous growth

had been made, the grass was clipped periodically over a period of several weeks, and the clippings were weighed. Table 6 gives the treatments, and the results in actual green weights of the grass clippings.

Series 4; Part 2: Grass

This series paralleled part 1, the same kind of soil and seed being used. A sample of sludge was obtained which had been subjected to the action of fungi in a closed box for several days before being dried and ground. The treatments and results are shown in part 2 of table 6.

It is apparent from the results of this series that dried, ground, fresh sewage sludge is of considerable value as a turf dressing, giving a better growth than an application of mineral fertilizer. The use of minerals as a supplement to the sludge, in the case of grass, would seem to be uneconomical. The fungus

TABLE 6

Grass

| POT NUMBER | TREATMENT | GREEN WEIGHTS |
|---------------|---------------------------------|---------------|
| | gm. | gm. |
| <i>Part 1</i> | | |
| 1 | 20 sludge | 28.00 |
| 2 | 20 sludge, 1.1 superphosphate | 23.15 |
| 3 | 20 sludge, 2.0 minerals (5-8-5) | 28.25 |
| 4 | 2 minerals (5-8-5) | 19.95 |
| 5 | Nothing (check) | 12.75 |
| <i>Part 2</i> | | |
| 6 | Nothing (check) | 12.25 |
| 7 | 20 sludge | 19.10 |
| 8 | 20 sludge (fungus treated) | 20.40 |

treatment of the sludge is shown by the experiment to be of no benefit in bringing the plant-food contained in the sludge into a more available form.

Series 5: Corn

Sweet corn was grown in Sassafras sand, 10 pounds of soil to the pot, and the moisture was maintained by periodic weighings and replenishments. The germination in all cases was excellent. Two weeks after planting, the plants were thinned to six a pot; then three and one-half weeks after planting three plants were removed from each pot and weighed; five and one-half weeks after planting two more were removed; and the last remaining plant was harvested nine weeks after planting. The treatments and the green weights, average for each plant of those removed from each pot, are given in table 7.

The heights were measured weekly, by drawing up the longest leaves, the results being shown in table 8. The apparent decrease in height of some of the plants during the last weeks of the experiment was due to the shriveling of the ends of some of the leaves, and not to a dying back of the crown of the plant. At this time upward growth had practically ceased, and the plants were in most cases in tassel, and some, for example those in pots 13, 14, and 16, were forming ears.

TABLE 7
Corn

| POT NUMBER | TREATMENT | GREEN WEIGHTS | | |
|------------|-----------------------|---------------|----------|---------|
| | | 3½ weeks | 5½ weeks | 9 weeks |
| | gm. | gm. | gm. | gm. |
| 1 | Nothing (check) | 3.81 | 8.38 | 12.85 |
| 2 | 2 lime | 3.33 | 6.25 | 13.75 |
| 3 | 2½ minerals (5-8-5) | 5.40 | 13.15 | 51.55 |
| 4 | 2½ minerals (5-8-5) | | | |
| | 2 lime | 5.39 | 10.40 | 29.25 |
| 5 | 45 sludge | 3.96 | 7.35 | 15.60 |
| 6 | 45 sludge, 2 lime | 3.32 | 7.18 | 12.78 |
| 7 | 90 sludge | 3.13 | 7.98 | 17.68 |
| 8 | 90 sludge, 2 lime | 4.02 | 6.28 | 13.15 |
| 9 | 45 sludge | | | |
| | ½ sulfate of ammonia | 4.88 | 8.05 | 20.10 |
| 10 | 45 sludge | | | |
| | 2½ sulfate of ammonia | 3.82 | 7.08 | 14.40 |
| 11 | 45 sludge | | | |
| | 2½ sulfate of ammonia | | | |
| | 2 lime | 4.10 | 9.03 | 18.25 |
| 12 | 45 sludge | | | |
| | 1½ superphosphate | 3.65 | 9.80 | 51.80 |
| 13 | 45 sludge | | | |
| | 5½ superphosphate | 4.05 | 10.80 | 68.80 |
| 14 | 45 sludge | | | |
| | 2½ sulfate of ammonia | | | |
| | 5½ superphosphate | 3.25 | 16.08 | 89.26 |
| 15 | 45 sludge | | | |
| | ½ muriate of potash | 3.96 | 10.15 | 16.15 |
| 16 | 45 sludge | | | |
| | 2½ minerals (5-8-5) | 5.05 | 14.33 | 81.80 |

The results of this experiment are, in general, similar to those already found in the preceding series. Medium or light applications of sludge alone are of practically no benefit, whereas heavy applications increase plant growth to some extent, as is shown by treatments 7 and 8.

The conclusion drawn from series 1, that a phosphate supplement is desirable, is further confirmed by treatments 12 and 13 of this series, and by the large

increase in growth of 14, which received sludge supplemented by superphosphate and sulfate of ammonia, over 10 and 11, which received the same

TABLE 8
Height of corn in inches

| POT NUMBER | WEEKS | | | | | | | | |
|---------------|-------|------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | 1.97 | 8.48 | 13.90 | 18.70 | 20.50 | 21.88 | 22.13 | 22.00 | 22.00 |
| 2 | 2.04 | 8.21 | 13.35 | 18.40 | 19.90 | 22.00 | 22.38 | 22.25 | 22.50 |
| 3 | 2.31 | 8.86 | 14.55 | 19.75 | 26.70 | 29.75 | 31.50 | 32.25 | 33.50 |
| 4 | 2.23 | 9.25 | 15.30 | 21.00 | 24.80 | 29.00 | 31.38 | 32.13 | 32.50 |
| 5 | 2.44 | 9.02 | 14.20 | 17.10 | 19.40 | 21.75 | 22.60 | 22.73 | 22.50 |
| 6 | 2.13 | 8.90 | 14.25 | 18.80 | 21.40 | 21.75 | 22.25 | 22.13 | 21.50 |
| 7 | 2.38 | 9.30 | 14.40 | 18.95 | 21.70 | 23.50 | 24.50 | 25.25 | 25.25 |
| 8 | 2.16 | 8.79 | 13.67 | 17.05 | 20.00 | 23.38 | 24.50 | 24.63 | 24.50 |
| 9 | 2.63 | 9.98 | 15.10 | 19.60 | 21.60 | 22.28 | 24.13 | 24.00 | 23.75 |
| 10 | 2.52 | 8.77 | 14.10 | 19.20 | 21.80 | 24.25 | 25.63 | 26.25 | 26.00 |
| 11 | 2.68 | 9.48 | 15.15 | 19.75 | 22.50 | 24.00 | 25.75 | 26.00 | 25.50 |
| 12 | 1.64 | 8.48 | 13.71 | 18.85 | 22.80 | 26.88 | 30.50 | 32.35 | 33.50 |
| 13 | 2.34 | 9.38 | 14.15 | 18.80 | 23.00 | 31.50 | 32.50 | 33.75 | 36.50 |
| 14 | 2.15 | 8.71 | 13.85 | 21.30 | 26.40 | 28.50 | 39.75 | 42.13 | 46.25 |
| 15 | 2.23 | 9.63 | 15.20 | 20.80 | 24.70 | 26.75 | 28.00 | 27.50 | 27.00 |
| 16 | 2.75 | 9.86 | 16.45 | 21.90 | 26.70 | 32.25 | 34.25 | 37.00 | 38.50 |

TABLE 9
Loss of moisture in pounds

| POT NUMBER | DATES | | | | | | | | | | TOTAL |
|---------------|-------|------|------|------|------|------|------|------|------|------|-------|
| | 12/31 | 1/10 | 1/18 | 1/22 | 1/26 | 2/2 | 2/8 | 2/14 | 2/20 | 2/27 | |
| 1 | 0.25 | 0.83 | 0.55 | 0.45 | 0.35 | 0.78 | 0.60 | 0.38 | 0.60 | 0.80 | 5.59 |
| 2 | 0.25 | 0.73 | 0.53 | 0.38 | 0.23 | 0.63 | 0.55 | 0.33 | 0.50 | 0.73 | 4.86 |
| 3 | 0.25 | 0.70 | 0.63 | 0.48 | 0.40 | 1.03 | 1.00 | 0.63 | 0.98 | 1.23 | 7.33 |
| 4 | 0.28 | 0.78 | 0.60 | 0.48 | 0.38 | 0.95 | 0.88 | 0.53 | 0.85 | 1.10 | 6.83 |
| 5 | 0.23 | 0.63 | 0.50 | 0.38 | 0.33 | 0.70 | 0.60 | 0.35 | 0.45 | 0.68 | 4.85 |
| 6 | 0.20 | 0.60 | 0.45 | 0.35 | 0.28 | 0.63 | 0.55 | 0.23 | 0.40 | 0.58 | 4.27 |
| 7 | 0.18 | 0.38 | 0.43 | 0.28 | 0.33 | 0.70 | 0.60 | 0.33 | 0.45 | 0.65 | 4.33 |
| 8 | 0.18 | 0.50 | 0.43 | 0.33 | 0.30 | 0.68 | 0.58 | 0.26 | 0.38 | 0.60 | 4.24 |
| 9 | 0.28 | 0.70 | 0.65 | 0.50 | 0.40 | 0.90 | 0.73 | 0.35 | 0.55 | 0.68 | 5.74 |
| 10 | 0.23 | 0.63 | 0.53 | 0.43 | 0.30 | 0.70 | 0.65 | 0.35 | 0.53 | 0.68 | 5.03 |
| 11 | 0.10 | 0.48 | 0.50 | 0.43 | 0.35 | 0.83 | 0.65 | 0.36 | 0.43 | 0.63 | 4.76 |
| 12 | 0.20 | 0.65 | 0.63 | 0.40 | 0.35 | 0.95 | 0.93 | 0.58 | 1.03 | 1.30 | 7.02 |
| 13 | 0.23 | 0.68 | 0.63 | 0.40 | 0.35 | 1.03 | 1.03 | 0.65 | 1.13 | 1.38 | 7.56 |
| 14 | 0.20 | 0.58 | 0.70 | 0.50 | 0.50 | 1.23 | 1.18 | 0.83 | 1.28 | 1.38 | 8.38 |
| 15 | 0.20 | 0.68 | 0.55 | 0.53 | 0.35 | 0.80 | 0.65 | 0.30 | 0.45 | 0.63 | 5.14 |
| 16 | 0.23 | 0.73 | 0.70 | 0.55 | 0.40 | 1.18 | 1.05 | 0.65 | 1.20 | 1.40 | 8.09 |

amounts of sludge and ammonia but no phosphate. The mixture in treatment 14 is equivalent on a ton basis to 1700 pounds of dried fresh sludge, 100 pounds

of sulfate of ammonia, and 200 pounds of 20 per cent superphosphate. It is applied at the rate of approximately 12 tons an acre.

The large differences between the heights and weights of the plants in pot 3 and those in pot 16, receiving minerals, and sludge and minerals, respectively, are especially noteworthy, inasmuch as the same mineral treatment was used in each case.

It should be borne in mind that the larger corn plants transpire very considerable quantities of water, and in interpreting table 9, it is necessary to refer to tables 7 and 8 and for comparison select treatments giving similar growth. Thus by comparing pot 1, receiving no sludge, with 5 receiving 45 gm. of sludge and 7 receiving 90 gm. of sludge, all of which show a comparable growth, we find that the loss of moisture is in the inverse order; namely, 1 lost 5.59 pounds, 5 lost 4.85 pounds, and 7 lost but 4.33 pounds. By calculating this to an acre basis, 1 lost 487,000 pounds of water an acre, 5 lost 422,532 pounds, and 7 lost 377,229 pounds. The difference between that lost by 7 and that lost by 1 is the equivalent of 0.5 inches of rainfall in the period of the experiment, which was nine weeks. This is of very considerable importance in a locality where the soil is so predominantly coarse sand.

This series has shown little benefit from the use of lime with the sludge. In fact where parallel treatments are used with and without lime, the lime has slightly depressed growth in all cases as measured by the heights of the plants, and in all but one case where measured by green weights.

DISCUSSION

From the results of the experiments herein reported, it would seem that dried fresh sewage sludge could be profitably employed as a fertilizer material, and especially as a soil builder. Although light applications did not appear to be of any benefit to plant growth, series 2 and series 5, beets and sweet corn, both heavy feeders, showed that when used at the rate of approximately 10 tons or more an acre, the dried fresh sludge will considerably increase plant growth without the use of any mineral fertilizer supplements.

As is to be expected from the nature of the material and its method of treatment, only the insoluble and hence slowly available nitrogen is left in the dried sludge. Because of the high content of carbonaceous material and this lack of available nitrogen, the decomposition of the sludge in the soil will deplete the supply of the available soil nitrogen at the expense of higher plants. The coincidental application of some nitrogen carrier such as nitrate of soda or sulfate of ammonia with the dried fresh sludge will therefore not only give the crop a quick start but will also increase the rapidity of the decomposition of the sludge.

The desirability of the use of a phosphate supplement in conjunction with dried fresh sewage sludge is indicated both by the analysis and by the experiments. A phosphate fertilizer is required under almost all soil conditions and for most crops, and since the sludge used was almost devoid of this plant-

food, it is not surprising that the supplementing of the sludge with superphosphate should give a large growth response.

Potash on the other hand, seldom gives as marked an increase in plant growth as nitrogen and phosphorus, and this has been the case in the experiments reported herein. While many soils contain a considerable quantity of potash, more-or-less available, it is very likely that potash will increase growth sufficiently on the sandy soil of the Atlantic City region to warrant its use in conjunction with the dried fresh sewage sludge.

These experiments would indicate, therefore, that to obtain the best and most efficient results from the use of dried fresh sludge, it should be supplemented with available nitrogen, phosphorus, and potash carriers. A mixture suggested by the results of the experiments would be 1650 pounds of dried, ground fresh sewage sludge, 100 pounds of sulfate of ammonia, 200 pounds of 20 per cent superphosphate, and 50 pounds of muriate of potash, to make a ton. The carbon-nitrogen ratio in such a mixture would be approximately 6, which is well within that found to be desirable by experiment (series 3).

As was pointed out in the discussion of series 4, grass, no mineral supplement appears to be necessary to give a good turf growth. It is well known that grass requires large amounts of moisture for satisfactory continuous growth. It is highly probable that the chief benefit from the use of dried fresh sludge on grass, especially where the soil is so extremely sandy, is the increasing of the water-holding capacity and the prevention of the rapid drying of the surface soil where the grass roots are concentrated. This has been clearly brought out in table 9.

Although the experiments have shown variable results from the use of lime with the sludge, it is quite certain that if this material were used on the same soil year after year, a considerable acidity would be developed, and lime would become necessary. This would be especially true if sulfate of ammonia were used in conjunction with the sludge.

It is of course freely admitted that in order to obtain thoroughly conclusive results and to determine what the action of dried fresh sewage sludge will be under varying soil and climatic conditions and its effect on large numbers of widely different types of plants, a series of experiments run continuously for several years would be necessary. It is felt however, that the experiments described in this paper have sufficiently proved that dried fresh sewage sludge has considerable fertilizer value when used under proper conditions. Nothing has been said regarding the mechanics or the economics of the grinding and drying processes, as that is an engineering problem.

SUMMARY

Several samples of dried fresh sewage sludge were analyzed, and pot experiments were carried out to determine the fertilizer value of this kind of material.

The analyses showed considerable potential plant-food to be present.

The narrowing of the carbon-nitrogen ratio to below 8 by the addition of available nitrogen largely increased the fertilizer value of the sludge.

A phosphate supplement appears to be necessary for good plant growth, and a potash supplement, in small quantities, would seem desirable.

The dried sludge alone, without mineral supplements of any kind, when applied to turf grown on sand gave a good stand of grass and prevented its dying off.

The application of dried fresh sludge to a sandy soil increased its water-holding capacity very materially, which is a most desirable result on soils of this type.

Although the experiments reported did not indicate a marked necessity of using lime with the sludge, it is almost certain that lime would be required after several years continuous application of sludge.

STUDIES OF NITROGEN FIXATION BY THE ROOT NODULE BACTERIA OF THE LEGUMINOSAE¹

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It is now more than forty years since Beijerinck published his memorable paper concerning the bacteria in the root nodules of the *Leguminosae*. During these four decades there have appeared in the scientific literature some 46 papers which deal to a greater or less extent with the problem of nitrogen assimilation by means of these nodule-forming bacteria. Although many careful studies have been made, it must be admitted that as yet no satisfactory evidence has been forthcoming to explain the part played by the bacteria in this assimilation of nitrogen by leguminous plants. The negative results, or almost negative, of so many investigators have been the stumbling block in the path of many bacteriologists. It is unfortunate that as yet no one has devised a satisfactory apparatus or medium which would greatly favor the assimilation of nitrogen by the bacteria in the absence of the host plant.² It may not be out of place to include a statement from Dible: "It is a natural characteristic of research workers in all countries that they publish their positive findings but often relegate their negative ones to oblivion." Such is not the case with this report. The results are mainly negative. Certain investigators report well-defined gains whereas others are unable to find any increase in combined nitrogen. In table 1 their findings are summarized. The column designated "Number of analyses," is an approximate estimate of the total number. The figures do not include any preliminary analyses. In the column "Nitrogen fixed," an effort was made to reduce all results to a common basis, milligrams of nitrogen in 100 cc., and in the last column on the right to give the author's conclusions regarding his results.

Many of these reports fail to give complete information regarding the medium, the cultures used, and the results of the analyses. These papers need not be discussed, inasmuch as the essential data are given in the table. In some cases there is doubt about the purity of the cultures. For example, there is little doubt that Mazé's cultures (35, 36) were impure because the author speaks of the "cheesy" odor of his cultures and because sufficient pre-

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

² Dible, J. H. 1929 Recent Advances in Bacteriology. Philadelphia.

TABLE 1

A summary of reports upon the fixation of atmospheric nitrogen by root nodule bacteria in culture

| | MEDIUM USED | NUMBER OF ANALYSES (ESTIMATED) | NITROGEN FIXED IN 100 CC. OF CULTURE | REPORT |
|--------------------------------|--|--------------------------------|--------------------------------------|-----------|
| | | | mgm. | |
| Beijerinck (4) | Nitrogen free salts, asparagin agar | | | No |
| Prazmowski (41) | Nitrogen-free medium | | | Yes |
| Beijerinck (5)* | Bean seedling extract + sucrose + KH_2PO_4 | 6 | 0.9-1.8 | Probable |
| Frank (13) | Nitrogen-free medium | | | Uncertain |
| Immendorff (30) | Various kinds | | None | No |
| Berthelot (7) | "Cohn's medium" + humic acid | | 5.3†† | Yes |
| Gonnermann (19) | Potato pulp | | None | No |
| Heinrich (25) | Potato pulp | 19 | None | No |
| Stutzer, Burri, and Maul (51)* | Potassium phosphate, MgSO_4 , NaCl , CaCl_2 , and glucose solution | 8 | 6.0 | No |
| Mazé (35)* | Bean seed extract + NaCl + NaHCO_3 + sucrose, with and without agar | 6 (?) | 23.4-27.1 | Yes |
| Zinsser (53) | Potassium phosphate, MgSO_4 , "sugar" solution | | None | No |
| Mazé (36)* | Bean seed extract + sucrose | 3 | 24.2-30.0 | Yes |
| Stoklasa (50) | | | None | No |
| Grieg-Smith (20) | Lupine leaf extract + KH_2PO_4 + CaCl_2 + agar | | | No |
| Grieg-Smith (21) | Various media, including Mazé's | | None | No |
| Neumann (38)* | Plant extract and peat extract | 9 | 4.4-49.9 | Yes |
| Hiltner and Störmer, (28) | Glucose, KH_2PO_4 , and peptone, asparagin or KNO_3 solutions | | None | No |
| Chester (00)§§ | K_2HPO_4 , NaCl , FeSO_4 , CaCO_3 , glucose agar | 8 | 0.6-2.5 | Yes |
| Lewis and Nicholson (33)† | Sucrose salt solutions and sucrose bouillon | 30 | 0-16.2 | Yes |
| Löhnis (34)‡ | Soil extract + glucose + K_2HPO_4 | | 2.8-3.6 | Yes |
| Moore (37)* | Maltose, MgSO_4 , potassium phosphate solution | 90 | 0.2-2.2 | Yes |
| Golding (17) | Bean and pea plant juices + sugars, K_2HPO_4 , NaCl , FeSO_4 , MnSO_4 , MgSO_4 , and succinic acid | 10 | 2.1-3.5 (with pure cultures.) | |
| Grieg-Smith (22)§ | Glucose, sodium phosphate solution + agar | 56 | 1.0-4.0 | Yes |

TABLE 1—Continued

| | MEDIUM USED | NUMBER OF ANALYSES (ESTI- MATED) | NITROGEN FIXED IN 100 CC. OF CULTURE | REPORT |
|------------------|--|--|--|--------|
| | | | mgm. | |
| Budinov (11)* | Dilute bean extract + sucrose | 2 | 4.1 | Yes |
| Bottomley (8) | KH ₂ PO ₄ , NaCl, CaCO ₃ , FeSO ₄ , mannite solution | 6 | 0.4 | Yes |
| de'Rossi (43)** | Bean seed and bean and vetch leaf extracts + sugars, NaCl, solutions or + gelatin or agar | 33 | -27.7-3.1 per 100 gm. | No |
| Fred (14)* | K ₂ HPO ₄ , MgSO ₄ , NaCl, Fe ₂ (SO ₄) ₃ , CaCl ₂ , MnSO ₄ , glucose solution | | 0.18-1.68 | Yes |
| Bottomley (9) | Same as Bottomley 1909 | | 1.8 | Yes |
| Golding (18) | | | | Yes |
| Fred (15)* | Same salts as Fred 1909-10 + maltose and sucrose + agar | 101 | 0.15-1.66 | Yes |
| Bottomley (10) | MgSO ₄ , potassium phosphate, maltose solution | | 2.0 | Yes |
| Grieg-Smith (23) | ? | | 3-5.6 | Yes |
| Spratt (46, 47)* | MgSO ₄ , potassium phosphate, sucrose solution | 8 | 2.5-3.5 | Yes |
| Herke (26) | Soil extract + K ₂ HPO ₄ + mannite | 37 | 0.14-1.2 | Yes |
| Olaru (40) | Mazé's bean extract + Mn. | 22 | 1.5-32.1 | Yes |
| Rocasolano (42)* | Mannitol solution + Mn. | 8 | 2.1-9.6 | Yes |
| Beijerinck (6) | K ₂ HPO ₄ , lime, glucose solution + garden soil; other media | | | No |
| Hills (27)† | MgSO ₄ , KH ₂ PO ₄ , NaCl, CaSO ₄ , CaCO ₃ , mannite agar | 46 | 0.15-3.5 | Yes |
| Joshi (32) | Soil extract + K ₂ HPO ₄ + mannite | | 0.8-2.0 | Yes |
| Singh (44)* | Soil and sucrose, K ₂ HPO ₄ , solution | 42 | 0.25-10.75 | Yes |
| Hutchinson (28a) | | | 2.5 | Yes |
| Voicu (52)* | Bean seed extract + sucrose + Boron | 45 | 2.4-3.5 | Yes |
| Hutchinson (29) | | | | Yes |
| Barthel (2)†† | KH ₂ PO ₄ , MgSO ₄ , NaCl, CaSO ₄ , KNO ₃ , FeCl ₃ , mannite solution | 8 | -0.21-0.39 | No |

TABLE 1—*Concluded*

| | MEDIUM USED | NUMBER OF ANALYSES (ESTI- MATED) | NITROGEN FIXED IN 100 CC. OF CULTURE | REPORT |
|--------------------------------------|--------------------------------------|--|--|--------|
| | | | mgm. | |
| Fred, Whiting, and Hastings (16)† | Soil extract + sucrose | 4 | 0.1 | Yes |
| Allison (1)* | Clover plant extract + glucose | | None | No |
| Bazarewski (3) | Synthetic medium | | 1.3-3.0 | Yes |
| Stiehr (48) | Lupine extract-glucose agar | 5 | 0.6-2.8 per 100 gm. | Yes |
| Højlversen (24)§ | Moore's dextrose-Ashby's solution | | 0.2-9.86 | Yes |
| Skinner (45)§ | Ashby's agar | 4 (P) | None | No |

When no note is made, the method of analysis was not designated.

* Kjeldahl.

† Gunning modified to include nitrates.

‡ Kjeldahl-Wilfarth.

§ Kjeldahl-Gunning.

** Kjeldahl-Jodlbauer.

†† Bristol-Page.

‡‡ 5.3 mgm. gained in total culture. Volume not given.

§§ Chester, F. D. 1904 Soil bacteria and nitrogen assimilation. Del. Agr. Exp. Sta. Bul. 66.

cautions were not taken to sterilize the air with which the cultures were aerated. In spite of this fact, Mazé's experiments are often quoted as proof that the root nodule bacteria are capable of fixing nitrogen outside of the host plant. What appear to be the best papers are briefly summarized below.

Beijerinck was the first to test the nitrogen-fixing ability of the root nodule bacteria. In 1888 (4), he found that bacteria from the nodules of *Vicia faba* fixed no nitrogen in a solution of salts to which asparagin had been added. Organisms from the nodules of *Cytisus laburnum* fixed no nitrogen on a solid medium. Again in 1891 (5), the nodule bacteria were grown in bean seedling extract to which sucrose (1.5 to 2 per cent) and varying amounts of KH_2PO_4 were added. Analyses made by the Kjeldahl method showed a slight gain in nitrogen (0.9 to 1.8 mgm. N in 100 cc.). The author concluded that nitrogen fixation was probable, but was not satisfied that his experiments had established fixation. A further report in 1918 (6) stated that only a very slight fixation had occurred in a synthetic medium. Plant extracts containing 2 per cent sucrose gave a slight "but not convincing" fixation of nitrogen. The author at that time concluded that nitrogen is not fixed by the root nodule bacteria. Beijerinck was very careful throughout this work to examine his cultures for purity, so that on this point one feels secure.

Findings similar to those of Beijerinck are reported by a number of investigators; namely, Immendorff (30), Gonnermann (19), Heinrich (25), Zinsser (53), Stoklasa (50), and Grieg-Smith (20, 21).

The following quotation from Nobbe and Hiltner (39) very clearly expresses the opinion of certain investigators on the question of nitrogen fixation by nodule bacteria:

"Trotz vielfacher Versuche ist es bisher nicht mit Sicherheit gelungen, durch Kultur des *Bacterium radicicola* in den verschiedensten Medien eine in Betracht kommende Zunahme des Stickstoffgehaltes zu erzielen."

Stutzer, Burri, and Maul (51) grew the nodule bacteria of alfalfa in a glucose mineral-salt solution and placed long-fibred asbestos in each flask. Although a gain in nitrogen of 6 mgm. in 100 cc. was found, no fixation was reported.

Soil extract with 1 per cent of glucose and 0.05 per cent K_2HPO_4 was used by Löhns (34) for the growth of bacteria of clover and vetch nodules. The soil extract contained no nitrates, but in order to determine the nitrates which he states were absorbed from the air, a nitrogen method was used to include the nitrate nitrogen, the Kjeldahl-Wilfarth procedure. Gains in nitrogen of 2.8 to 3.6 mgm. in 100 cc. were found.

Moore (37) reports gains of nitrogen of 0.2 to 2.2 mgm. in 100 cc. of medium with red clover, soybean, white lupine, hairy vetch, berseem, and garden pea root nodule bacteria. A nitrogen-free solution of magnesium sulfate, potassium phosphate, and maltose was used, and the cultures were aerated.

Gains of like amounts of nitrogen are reported by Grieg-Smith (22), Bottomley (9, 10), Spratt (46, 47), Joshi (32), Hutchinson (28a), Bazarewski (3), and Stiehr (48).

Golding's work (17) is usually quoted with that of Mazé as proof that the root nodule bacteria fix atmospheric nitrogen. Golding attempted to remove the soluble products, by growing his cultures in an inverted and covered bell-jar, and drawing off the culture medium through a porous filter-candle at the bottom. Apparently the medium was not added continuously, and thus the conditions of the experiment were quite different from those existing in the plant.

In one experiment unheated, macerated young bean plants were placed in the inverted bell-jar with distilled water, and the apparatus attached to the suction pump. A gain of 11.4 mgm. of nitrogen in 100 cc. took place in 15 days. In later experiments the medium was sterilized and inoculated with pure cultures of root nodule bacteria (strain not mentioned). The gain in nitrogen amounted to about 2.1 and 3.5 mgm. of nitrogen in 100 cc.

De'Rossi (43) attempted to duplicate the results of Mazé, but the gains of nitrogen in 100 gm. of agar medium were: + 1.0 mgm., - 27.7 mgm., + 2.0 mgm. Other results were + 3.1 mgm., - 7.7 mgm. An experiment was also set up similar to that of Golding but showed the following results: + 4.2 mgm., - 3.4 mgm. N in 100 cc. This worker concluded that the nodule bacteria fix only insignificantly small amounts of nitrogen.

Olaru (40) used Mazé's medium for the growth of the root nodule bacteria, but added varying amounts of manganese salts. When no manganese was present in the medium, a fixation of 2.0 mgm. of nitrogen in 100 cc. took place,

but in one case when 0.5 mgm. of manganese was present, the gain in nitrogen was 32.1 mgm. Rocasolano (42) also reports that manganese has a stimulating effect on nitrogen fixation.

The effect of nitrates on the fixation of nitrogen by the nodule bacteria was studied by Hills (27). Varying amounts of calcium, potassium, and sodium nitrates were added to the agar. The nitrogen was determined by the modified Gunning (salicylic acid) method to include the nitrates. A fixation of 0.15 to 3.5 mgm. of nitrogen in 100 cc. is reported. However, the salicylic acid method has been found by Davisson and Parsons (12) to be unreliable for the determination of nitrate-nitrogen in the presence of water.

Barthel (2) obtained no fixation by pea organisms in a nitrogen-free mineral salts solution with and without caffeine added. Red clover plant extract, according to Allison (1), gave no gain in nitrogen when inoculated with red clover nodule bacteria.

In dextrose- Ashby's solution cultures of *Rh. leguminosarum*, Halversen (24) reported gains of 0.2 to 9.86 mgm. in 100 cc.

From the lack of agreement shown in the work cited, it is evident that the question of the fixation of nitrogen by the root nodule bacteria outside of the host plant is by no means settled. Many of the discrepancies can be explained on the basis of impure cultures, or method of analysis, but the variable factors such as the stimulating effect of certain chemical elements, the influence of the age of the inoculum, the differences in the amounts of nitrogen supplied the bacteria, the possible stimulating effect of plant extracts, do not admit the reduction of all these results to a common basis.

It was, then, in the hope that further experiments in which better methods were used would bring order out of the chaos, that this problem was undertaken.

EXPERIMENTAL

Preparation of media

Usually, a soil high in organic matter was heated for half an hour at 120°C. with an equal weight of water. Calcium carbonate (10 gm. per kilo of soil) was stirred into the hot soil sludge, and the liquid filtered from the soil. To the clear, pale yellow extract, 1 per cent of sugar (glucose or sucrose) and 0.05 per cent K_2HPO_4 were added. One hundred cubic centimeters of this medium was put into 750 cc. Erlenmeyer flasks, the flasks plugged with cotton and sterilized.

Culture methods

The cultures used for the inoculation were grown on slants of yeast water mannitol agar, and were 3 to 6 days old when used. The controls were inoculated in the same way as the culture flasks and were then heated for 10 to 25 minutes at 120°C., in an autoclave. The purity of all cultures used was tested before analysis.

Cultures were incubated at room temperature (20 to 25°C.) unless otherwise stated. Both young and old cultures were analyzed to determine the influence of age on the fixation of nitrogen.

Methods of analysis

The nitrates, which were invariably found present in the soil extracts, made necessary the use of a method of analysis which would include this form of nitrogen as well as the organic and ammonia nitrogen. If the nitrate nitrogen were lost in analysis, the nitrogen present in this form in the controls would be driven off, whereas in the inoculated cultures the nitrates might have been used by the bacteria and converted into protein. An apparent fixation would be the result of such an occurrence. The salicylic acid method (Gunning method modified to include nitrates) had been shown by Davisson and Parsons (12) and by Jacob and Geldard (31) to be unreliable for the determination of nitrates in the presence of water. The Davisson-Parsons method was used, as it appeared to be the most reliable and convenient method of nitrogen determination to include nitrates.

Titration were made with $1/28 N$ NaOH and H_2SO_4 .

Experiment 1

In 1927 a number of experiments dealing with the problem of nitrogen fixation by the nodule bacteria in pure culture were made by Miss M. P. Löhnis. As a culture medium, among others, soil extract with mannitol or glucose added was used, and the nitrogen analyses were made by the Gunning method modified to include nitrates. A large number of positive results were obtained, and those in which soil extract was used are given in table 2.³ The cultures studied included Dalea 901, Bean 402, Red Clover 205, Sweet Clover 115, Soybean 501, and Pea 317 and 302. A fixation of -0.27 to $+2.13$ mgm. of nitrogen in 100 cc. seems to have taken place. All cultures except Soybean 501 showed a small gain of nitrogen. One set of cultures of Red Clover 205 and Pea 317 were aerated with air passed through cotton, acid, and alkali towers. The apparent fixation as shown in the figures of this table is, no doubt, due to the faulty method of analysis.

As the soil extract contained nitrates, it was deemed necessary to check these results with the more reliable method of Davisson-Parsons.

Experiment 2

The total nitrogen of the soil extract prepared by the method described in the foregoing was determined by the Davisson-Parsons method. Although copper is present in the Devarda's alloy used in this method for the reduction of the nitrates, it was feared that insufficient amounts were present to catalyze

³ A more detailed description of these experiments will be published elsewhere.

completely the oxidation of the organic matter. Three 100-cc. portions of soil extract were analyzed by the Davisson-Parsons method, and to three others 0.7 gm. HgO was added, the procedure being otherwise identical with that recommended by Davisson and Parsons. Triplicate analyses made in

TABLE 2
The gain and loss of nitrogen by soil extract cultures of root nodule bacteria

| ORGANISM | NUMBER OF CULTURES SHOWING | | GAIN OR LOSS IN NITROGEN | |
|-----------------------|----------------------------|------------------|--------------------------|------------------|
| | Gain in N | Loss in N | Maximum | Average |
| | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. | mgm. per 100 cc. |
| Pea 317..... | 16 | 2 | 2.26 | 1.06 |
| Pea 302..... | 2 | 0 | 1.13 | 0.99 |
| Red Clover 205..... | 18 | 0 | 3.42 | 1.75 |
| Bean 402..... | 12 | 2 | 3.19 | 1.38 |
| Sweet Clover 115..... | 7 | 3 | 2.40 | 0.74 |
| Soybean 501..... | 3 | 6 | 2.00 | -0.27 |
| Dalea 901..... | 17 | 0 | 3.33 | 2.13 |

TABLE 3
Analyses of cultures of Dalea 901 in soil extract
(Second experiment. Age 11 days)

| CONTROL NUMBER | TOTAL NITROGEN | CULTURE NUMBER | TOTAL NITROGEN |
|----------------|------------------|----------------|------------------|
| | mgm. per 100 cc. | | mgm. per 100 cc. |
| 1 | 2.1 | 37 | 2.2 |
| 2 | 2.1 | 38 | 2.1 |
| 3 | 2.3 | 39 | 2.4 |
| 4 | 2.3 | 40 | 2.1 |
| 5 | 2.4 | 42 | 1.6 |
| 6 | 2.3 | 43 | 1.9 |
| 7 | 2.1 | 44 | 1.7 |
| 8 | 2.0 | 45 | 2.0 |
| | | 46 | 2.1 |
| | | 47 | 2.1 |
| | | 48 | 2.1 |
| | | 49 | 2.0 |
| | | 50 | 2.0 |
| | | 51 | 2.0 |
| | | 52 | 1.9 |
| Average..... | 2.2 | | 2.0 |

these two ways gave identical results, indicating that HgO was not necessary. The total nitrogen content of this soil extract averaged 2.1 mgm. per 100 cc.

The nutrient materials, K_2HPO_4 and glucose, were added to the soil extract and 100-cc. portions of the medium sterilized in 750-cc. Erlenmeyer flasks. These flasks were inoculated with three drops of a suspension of 3-day-old

root nodule bacteria of known pure cultures. The organisms used in this experiment were: Dalea 901, Alfalfa 100, and Red Clover 205.

The flasks were incubated at room temperature. Eleven days after being inoculated, all Dalea 901 cultures were tested for purity, and they, with eight controls, were analyzed for total nitrogen. The analytical results are given in table 3. Apparently, a slight loss of nitrogen had taken place.

Sugar analyses by the micro method of Stiles, Peterson, and Fred (49) of 35-day-old cultures of Red Clover 205 and Alfalfa 100 indicated that very little fermentation (5 to 9 per cent) had taken place. This was no doubt due to the unfavorable reaction of the medium, which was pH 4.6 to 5.2. These cultures failed to gain in nitrogen. In view of the small amount of sugar fermented, the results are not included in the tables.

Experiment 3

In order to obtain a soil extract higher in nitrogen content than that used in the last experiment, the soil-water mixture was heated for 50 minutes at 120°C. This additional heating of 20 minutes resulted in an increase in the soluble nitrogen of the soil extract of about 1 mgm. in 100 cc. Glucose and K_2HPO_4 were added to the soil extract, and the medium was sterilized. As in the previous experiment, it was found to be difficult to maintain a reaction in soil extract favorable for the growth of the nodule bacteria. The pH of the soil extract in this experiment was 7.6 before sterilization and 6.0 after. It was adjusted to pH 6.8 with sterile alkali before inoculation. Five-day-old cultures of the following organisms were used to inoculate the soil extract medium. Alfalfa 100, Red Clover 205 and 202, Pea 310 and 311, and Dalea 901. The cultures were incubated at room temperature, and were shaken on alternate days.

Fifty-three days after inoculation, five flasks of each culture were analyzed for total nitrogen. See table 4 for the results of these analyses, and also of those made when the cultures were 60 days old. For the loss of 0.3 to 0.4 mgm. of nitrogen by the Alfalfa 100 cultures no explanation is offered. No fixation in amounts beyond experimental error is shown by any of these cultures.

Experiment 4

The inconstancy of the reaction of the media in previous experiments made it seem probable that insufficient growth of the bacteria had taken place to give an appreciable fixation of nitrogen. More K_2HPO_4 was then used in this experiment than had been used previously; the amount was raised from 0.05 per cent to 0.1 per cent and in addition about 5 gm. of $CaCO_3$ added to one-half of the flasks. Sucrose, because it is much more stable to alkali, was substituted for glucose. The pH of this soil extract medium at the beginning was 7.4 and this was unchanged by sterilization. The addition of 5 cc. of 0.1 *N* HCl caused the reaction to drop to only 6.4. The medium is thus

TABLE 4

The total nitrogen, the percentage of glucose fermented, and the reaction of the cultures
(Fourth experiment)

| ORGANISM | NITROGEN ANALYSES AFTER | | GLUCOSE FERMENTED* | REACTION |
|----------------------|-------------------------|------------------------|-----------------------|-----------|
| | 53 days | 60 days | | |
| | <i>mgm. in 100 cc.</i> | <i>mgm. in 100 cc.</i> | <i>per cent</i> | <i>pH</i> |
| Control. | 3.9 | 3.8 | | 5.2 |
| | 3.8 | 3.7 | | |
| | 3.8 | 3.7 | | |
| | 3.8 | 3.9 | | |
| | 3.8 | Av. 3.8 | | |
| | Av. 3.8 | | | |
| Red Clover 205. | 3.9 | 3.8 | 23.8 | 4.4 |
| | 3.8 | 3.8 | | |
| | 4.0 | 3.7 | | |
| | 4.0 | Av. 3.8 | | |
| | 4.0 | | | |
| | Av. 3.9 | | | |
| Red Clover 202. | 3.8 | 3.7 | 16.8 | 4.4 |
| | 3.8 | 3.9 | | |
| | 3.8 | 3.8 | | |
| | 3.8 | Av. 3.8 | | |
| | Av. 3.8 | | | |
| Alfalfa 100. | 3.8 | 3.7 | 20.8 | 5.2 |
| | 3.2 | 3.2 | | |
| | 3.8 | 3.4 | | |
| | 3.2 | Av. 3.4 | | |
| | 3.3 | | | |
| | Av. 3.5 | | | |
| Pea 310. | 4.0 | 3.9 | 25.3 | 4.2 |
| | 4.2 | 3.8 | | |
| | 4.4 | Av. 3.9 | | |
| | 3.8 | | | |
| | Av. 4.1 | | | |
| Pea 311. | 3.8 | | 10.4 | 4.6 |
| | 3.8 | | | |
| | 3.9 | | | |
| | Av. 3.8 | | | |
| | | | | |
| Dalea 901. | 4.1 | 4.0 | 12.5 | 4.6 |
| | 4.3 | 4.0 | | |
| | 4.3 | Av. 4.0 | | |
| | 4.1 | | | |
| | 4.1 | | | |
| | Av. 4.2 | | | |

* Sugar and pH determinations after 35 days.

buffered sufficiently to maintain a reaction favorable for the growth of the root nodule bacteria, even though considerable acid is produced. Each of the following cultures were grown both with and without CaCO_3 : Pea 310, Alfalfa 100, Red Clover 205, and Soybean 504. The flasks were inoculated with 6-day-old cultures.

TABLE 5

The total nitrogen according to two methods of analysis, percentage of sugar fermented and presence or absence of nitrates in cultures

(Fourth experiment)

| ORGANISM | TOTAL NITROGEN | | NITRATES | GLUCOSE FER- MENTED |
|----------------------------------|--|--------------------------------------|----------|------------------------|
| | Davison-Parsons | Gunning modified | | |
| | mgm. in 100 cc. | mgm. in 100 cc. | | |
| <i>Without calcium carbonate</i> | | | | |
| Control | 4.8, 4.8, 4.7, 4.7, 4.8, 4.9, Av. 4.8 | 4.3, 4.3, 4.3, 4.2, Av. 4.3 | + | — |
| Red Clover 205 | 4.8, 4.8, 4.7, 4.7, 4.6, Av. 4.7* | 5.1, 5.2, 5.2, 5.2, 5.6, Av. 5.3* | — | 25.7 |
| Pea 310 | 4.8, 4.9, 4.9, 5.3, 5.1, Av. 5.0* | 5.3, 5.6, 5.8, 5.7, 5.7, Av. 5.6* | — | 36.0 |
| Alfalfa 100 | 4.1, 4.2, 4.1, 4.1, Av. 4.1 | 4.2, 4.1, 4.1, 4.1, 4.0, Av. 4.1 | — | 12.9 |
| Soybean 504 | 4.2, 4.7, 4.3, 4.2, 4.3, Av. 4.3 | 4.2, 4.1, 4.2, 4.1, 4.4, Av. 4.2 | — | None |
| <i>With calcium carbonate</i> | | | | |
| Control | 4.7, 4.7, 4.8, 4.2, Av. 4.6 | 4.8, 4.1, 4.0, 4.1, Av. 4.3 | + | — |
| Red Clover 205 | 5.0, 4.9, 4.9, Av. 4.9 | 5.0, 5.4, 5.5, 5.6, Av. 5.4 | — | 12.1 |
| Pea 310 | 5.1, 5.2, 4.7, Av. 5.0 | 5.6, 5.5, 5.6, Av. 5.6 | — | 29.8 |
| Alfalfa 100 | 4.5, 4.1, 5.1, 4.5, Av. 4.6 | 4.4, 4.2, 4.1, 4.7, Av. 4.4 | + | None |
| Soybean 504 | 4.2, 4.8, Av. 4.5 | 3.9, 4.3, 4.2, 4.1, Av. 4.1 | + | 1.6 |

* Red clover and pea cultures without CaCO_3 developed an acid reaction, pH 6.0 and 6.6.

According to Nobbe and Hiltner (39) it is the bacteroid-form which fixes the nitrogen in the nodules of leguminous plants. If this is the case, bacteroids might be expected to fix nitrogen outside of the host plant. In stained mounts from our cultures, branched forms were observed in the pea, alfalfa, and red clover cultures, and small rods with two deeply staining bodies in the soybean cultures after 14 days of incubation. After 23 days incubation, branched forms were observed in all four cultures, with and without CaCO_3 .

Sugar analyses, pH readings, and nitrate tests were made on 32 to 34-day-old cultures. These results are given in table 5. The cultures were very irregular in their growth. The alfalfa culture without calcium carbonate had fermented very little sugar (13 per cent), whereas the culture with calcium carbonate did not show any loss of sugar. The soybean cultures with no calcium carbonate, likewise did not show any loss of sugar. Although growth was evident in these cultures no appreciable quantity of sugar was fermented. The analyses on these cultures are included as further checks on the controls.

Five flasks from each series were analyzed for total nitrogen by the Davisson-Parsons method when the cultures were 25 to 26 days old, and the same number at the age of 52 days were analyzed by the Gunning method modified to include nitrates. These results are given in table 5. The cultures analyzed by the Davisson-Parsons method again show no fixation beyond the limit of experimental error. Alfalfa 100 and Soybean 504 cultures appear to have

TABLE 6
Nitrogen analyses of cultures 59 days old with colloidal soil added
(Fifth experiment)

| ORGANISM | FLASK 1 | FLASK 2 | FLASK 3 | FLASK 4 | FLASK 5 | FLASK 6 | FLASK 7 | AVER- AGES |
|---------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> | <i>mgm. per 100 cc.</i> |
| Controls..... | 4.8 | 4.8 | 4.8 | 4.9 | 4.8 | 4.9 | 4.8 | 4.8 |
| Pea 310..... | 5.0 | 5.1 | 5.1 | 5.0 | 4.9 | 4.9 | ... | 5.0 |
| Red Clover 205..... | 5.1 | 4.7 | 4.7 | 4.8 | 4.8 | Lost | ... | 4.8 |
| Soybean 504..... | 4.3 | Lost | 5.0 | 4.3 | 4.3 | Lost | ... | 4.5 |
| Alfalfa 100..... | 4.0 | 4.1 | 3.8 | 3.9 | 4.2 | 3.8 | ... | 4.0 |

lost nitrogen. In the series without CaCO_3 , the modified Gunning method analyses, on the other hand, show a fixation of nitrogen by Red Clover 205 (1.2 mgm. in 100 cc.) and Pea 310 (1.5 mgm. in 100 cc.) Alfalfa 100 and Soybean 504 show no gain in nitrogen, but check with the controls. The series to which CaCO_3 had been added, yielded similar results. It is evident then that the method of analysis is very important. In one of the papers, the modified Gunning method has been used for *culture solutions containing nitrates*, and fixation reported. These results are thus invalid.

Experiment 5

A soil extract was made up like that in the previous experiment (0.1 per cent K_2HPO_4 and 1 per cent sucrose). A colloidal suspension of soil was made to test the effect of colloids upon the nodule bacteria. One gram of water-extracted, dried soil was ground to 100 mesh, suspended in 50 cc. of water and run through a rotary lower plate type colloid mill. One cubic centimeter of this colloidal suspension was added to each flask containing 100 cc. of soil

extract. The flasks were plugged, sterilized, and inoculated in the same way as before, Red Clover 205, Pea 310, Soybean 504, and Alfalfa 100 being used for the experiment. The cultures were incubated at room temperature; 5 days after inoculation, stained mounts were made from representative flasks. Branched forms were observed in all the mounts.

Fifty-nine days after inoculation, analyses of the cultures were made by the Davisson-Parsons method. Table 6 gives the results. No fixation of nitrogen had taken place. In fact, cultures of Soybean 504 and Alfalfa 100 again showed a loss in nitrogen.

SUMMARY

Pure cultures of Alfalfa 100, Bean 402, Dalea 901, Pea 302, 310, 311, and 317, Red Clover 202 and 205, Soybean 501 and 504, and Sweet Clover 115 were grown in a soil extract containing 1 per cent sugar (glucose or sucrose) and 0.05 to 0.1 per cent K_2HPO_4 . From 5.4 to 36.0 per cent of the sugar was destroyed under the conditions of these experiments. The cultures were all tested for purity by the litmus milk and potato tests, and all impure cultures discarded. Soil extract cultures when analyzed by the Gunning method modified to include nitrates showed a fixation of as high as 2.5 mgm. of nitrogen to 100 cc. Analyses made of parallel flasks by the Davisson-Parsons method, which is more reliable for the determination of total nitrogen to include nitrate nitrogen, gave evidence of no fixation in amounts beyond the limit of experimental error. Alfalfa 100 and Soybean 501 and 504 consistently gave a loss of 0.1 to 1.7 mgm. of nitrogen to 100 cc.

From more than 500 total nitrogen analyses, it is obvious that nitrogen is not fixed by any of these 12 different cultures of Rhizobia under the conditions prevailing in these experiments.

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NITRATE CHANGES IN A FERTILE SOIL AS INFLUENCED BY SODIUM NITRATE AND AMMONIUM SULFATE¹

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INTRODUCTION

The changes that nitrogen salts undergo when added to soil have attracted the attention of many investigators, and the following workers, Lipman and Blair (5), Coleman (1), Russell (8), and others, have presented extensive reviews on the subject. Nitrate as a form of nitrogen for the plant is very efficient (11), and other nitrogenous compounds are converted to nitrates as a result of microbial activities (9). These activities are influenced by many factors, including temperature (4), moisture (6), aeration (3), soil reaction (12), and energy source (10).

Previous investigations have been conducted with a substrate consisting of sand, or of soil in need of nitrogen. For the experiment herein reported, however, a virgin Sassafras loam having a nitrogen content of 100 mgm. of nitrogen to 100 gm. of soil, and therefore showing no deficiency of nitrogen was employed. Varying amounts of NaNO_3 or $(\text{NH}_4)_2\text{SO}_4$ were added, and the nitrates were determined periodically, by the phenoldisulfonic acid method, the results being presented on the oven-dry basis. All analyses were run on a fresh sample of soil.

EXPERIMENTAL

Accumulation of nitrates in incubated soils

Tumblers each containing 100 gm. of soil were treated in duplicate as follows: (a) soil left untreated; (b) 2.5 mgm. of nitrogen added as NaNO_3 ; and (c) 2.5 mgm. of nitrogen added as $(\text{NH}_4)_2\text{SO}_4$. These salts were applied from a standard solution, then distilled water was added to the optimum moisture content, which was maintained by the addition of a few drops of

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water whenever necessary. The soils were incubated at a temperature of 27 to 30°C.

Periodically, the soil in each tumbler was thoroughly mixed, weighed, and an aliquot portion equivalent to 10 gm. of dry soil was removed for a nitrate determination. The results are given in table 1.

Although the incubation has increased the amounts of nitrate in the soil, it is noticeable that the treated soils show an increase in excess of the nitrogen added at only the 63-day, 119-day, and 133-day periods. The remainder of the analyses show a diminution of the nitrates. The values shown in table 1 are obtained by deducting the $\text{NO}_3\text{-N}$ found in the untreated soils from the amounts found in the nitrogen-treated soils, and then noting whether the differences are larger or smaller than 2.5 mgm. (the amount of nitrogen originally added).

The total $\text{NO}_3\text{-N}$ accumulation and the increase and decrease of the $\text{NO}_3\text{-N}$ in comparison to the added nitrogen are shown in figure 1.

TABLE 1
Nitrate-nitrogen accumulating in incubated soils

| TREATMENT | NITRATE-NITROGEN ACCUMULATED IN 100 GM. SOIL IN TIME INDICATED | | | | | | | | | |
|---|--|------------|------------|------------|------------|------------|------------|-------------|-------------|-------------|
| | 0 day | 21 days | 35 days | 49 days | 63 days | 77 days | 91 days | 105 days | 119 days | 133 days |
| | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| Untreated soil..... | 2.0 | 3.4 | 4.2 | 5.0 | 6.3 | 4.9 | 8.3 | 8.4 | 7.2 | 6.4 |
| 2.5 mgm. N added as NaNO_3 | 4.5 | 5.8 | 6.3 | 5.1 | 10.3 | 6.4 | 9.0 | 9.8 | 11.8 | 9.7 |
| 2.5 mgm. N added as $(\text{NH}_4)_2\text{SO}_4$ | 2.0 | 5.2 | 6.0 | 5.9 | 9.0 | 7.4 | 8.7 | 10.0 | 12.7 | 9.7 |

Nitrates leached

A series of lysimeters with a capacity for 1000 gm. of soil were prepared from glazed pots, each of which had an opening in the bottom. Into each opening was fitted a one-hole stopper carrying a glass tube to which was attached a piece of rubber tubing. These pots were then placed on a bench, through which the glass tubes penetrated.

For each treatment four pots of soil were prepared for a greenhouse experiment. Two of these pots were left fallow, and in each of the other two, six grains of oats were planted. Distilled water was added whenever it was necessary, to maintain optimum moisture conditions. At the periods indicated in table 2 and 3 the soils were leached with distilled water until the presence of nitrates was no longer detected by means of diphenylamine, and the $\text{NO}_3\text{-N}$ was then determined in the leachings.

Table 2 shows that a part of the $\text{NO}_3\text{-N}$ originally present in the soil and also a part of the added nitrogen have been changed to a form not readily

leached from the soil. This "fixed" nitrogen is later recovered as nitrates in the periodical leachings. Although a storing-up or a conversion of the nitrogen occurs, the addition of the nitrogenous salts neither inhibits nor accelerates the formation of nitrates from the original soil nitrogen. From the untreated soil 12.6 mgm. of $\text{NO}_3\text{-N}$ were leached in excess of the amount present (20 mgm.) at the beginning of the experiment, and values approximating this are

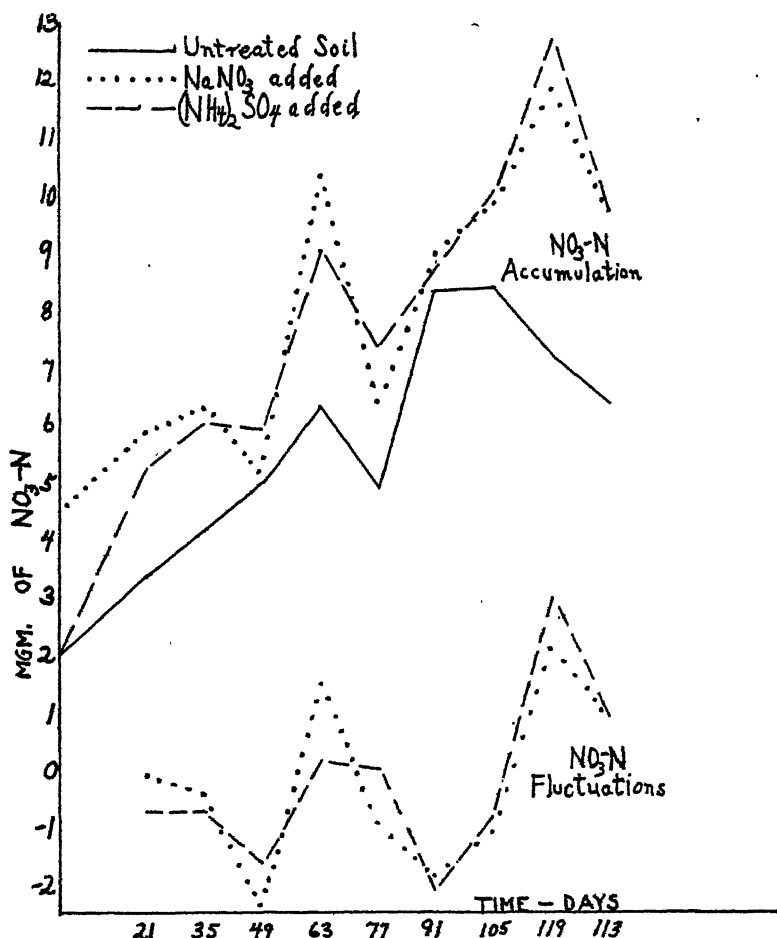


FIG. 1. NITRATE TRANSFORMATIONS IN 100 GM. INCUBATED SOILS

obtained in the NaNO_3 -treated soils. In the $(\text{NH}_4)_2\text{SO}_4$ -treated soils, slightly smaller quantities of nitrates are recovered, which may be due to the washing away of some of the ammonium salts.

Table 3 indicates that the oat plants diminish the amount of $\text{NO}_3\text{-N}$ leached from the soils. After the 70-day period, the plants utilize all of the nitrogen

made available; however, this was not sufficient for proper plant development, as was indicated by their growth.

TABLE 2
Nitrate-nitrogen leached from soil kept fallow

| TREATMENT | NITRATE-NITROGEN LEACHED FROM 1,000 GM. SOIL IN TIME INDICATED | | | | | | | | | |
|---|--|---------|---------|---------|---------|---------|---------|----------|----------|---------------|
| | 14 days | 28 days | 42 days | 56 days | 70 days | 84 days | 98 days | 112 days | 126 days | Total leached |
| | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| Untreated soil..... | 5.7 | 1.7 | 2.9 | 3.3 | 3.2 | 3.0 | 4.9 | 3.9 | 4.0 | 32.6 |
| Soil + 25 mgm. N as NaNO ₃ | 23.0 | 7.0 | 5.7 | 3.6 | 4.0 | 3.7 | 3.3 | 5.1 | 2.6 | 58.0 |
| Soil + 25 mgm. N as (NH ₄) ₂ SO ₄ | 16.4 | 7.1 | 4.6 | 3.7 | 4.1 | 2.9 | 3.0 | 4.8 | 2.8 | 49.4 |
| Soil + 50 mgm. N as NaNO ₃ | 40.1 | 11.8 | 7.8 | 3.5 | 4.9 | 3.5 | 3.2 | 3.7 | 3.5 | 82.0 |
| Soil + 50 mgm. N as (NH ₄) ₂ SO ₄ | 23.2 | 17.5 | 13.1 | 5.6 | 4.0 | 5.3 | 4.7 | 4.5 | 2.8 | 80.7 |

TABLE 3
Nitrate-nitrogen leached from soil planted to oats

| TREATMENT | NITRATE-NITROGEN LEACHED FROM 1,000 GM. SOIL IN TIME INDICATED | | | | | | | | | |
|---|--|---------|---------|---------|---------|---------|---------|----------|----------|---------------|
| | 14 days | 28 days | 42 days | 56 days | 70 days | 84 days | 98 days | 112 days | 126 days | Total leached |
| | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| Untreated soil..... | 4.3 | 0.3 | 0.1 | 0.1 | tr. | tr. | tr. | tr. | tr. | 4.8 |
| Soil + 25 mgm. N as NaNO ₃ | 13.6 | 1.6 | 0.1 | 0.1 | tr. | tr. | tr. | tr. | tr. | 15.2 |
| Soil + 25 mgm. N as (NH ₄) ₂ SO ₄ | 12.8 | 1.2 | 0.1 | 0.1 | tr. | tr. | tr. | tr. | tr. | 14.2 |
| Soil + 50 mgm. N as NaNO ₃ | 26.8 | 1.8 | 0.1 | 0.1 | tr. | tr. | tr. | tr. | tr. | 28.8 |
| Soil + 50 mgm. N as (NH ₄) ₂ SO ₄ | 17.6 | 6.5 | 0.3 | 0.1 | tr. | tr. | tr. | tr. | tr. | 24.5 |

TABLE 4
Nitrogen recovered in plants and NO₃-N not recovered from 1,000 gm. soil

| | UNTREATED SOILS | 25 MGL. N AS NaNO ₃ | 25 MGL. N AS (NH ₄) ₂ SO ₄ | 50 MGL. N AS NaNO ₃ | 50 MGL. N AS (NH ₄) ₂ SO ₄ |
|---------------------------|-----------------|--------------------------------|--|--------------------------------|--|
| | mgm. | mgm. | mgm. | mgm. | mgm. |
| N in plants..... | 20.4 | 24.9 | 23.9 | 29.9 | 31.2 |
| N rendered insoluble..... | 27.7 | 42.4 | 35.0 | 53.2 | 56.1 |
| N not accounted for..... | 7.3 | 17.5 | 11.1 | 23.3 | 24.9 |

At maturity (126 days) the entire plants were analyzed for nitrogen by the Kjeldahl method. The amount of nitrogen thus found in the plants and the amounts rendered insoluble in the planted pots are presented in table 4. This insoluble nitrogen is the amount of $\text{NO}_3\text{-N}$ leached from the planted pots,

TABLE 5
 $\text{NO}_3\text{-N}$ in 1,000 gm. fallow soil and soil planted to oats

| TREATMENT | 28 DAYS | 56 DAYS | 84 DAYS |
|----------------------------------|---------|---------|---------|
| | mgm. | mgm. | mgm. |
| Untreated soil: | | | |
| Fallow..... | 43.5 | 35.7 | 44.0 |
| Planted..... | 22.1 | 15.5 | 11.8 |
| 25 mgm. $\text{NO}_3\text{-N}$: | | | |
| Fallow..... | 61.0 | 52.6 | 71.6 |
| Planted..... | 39.8 | 22.4 | 27.8 |
| 25 mgm. $\text{NH}_4\text{-N}$: | | | |
| Fallow..... | 23.0 | 55.0 | 71.0 |
| Planted..... | 22.1 | 15.0 | 20.0 |
| 50 mgm. $\text{NO}_3\text{-N}$: | | | |
| Fallow..... | 67.2 | 88.9 | 105.8 |
| Planted..... | 63.8 | 47.0 | 53.9 |
| 50 mgm. $\text{NH}_4\text{-N}$: | | | |
| Fallow..... | 49.2 | 55.5 | 99.0 |
| Planted..... | 32.9 | 30.1 | 30.8 |

TABLE 6
Nitrogen recovered in plants

| TREATMENT | 28 DAYS | 56 DAYS | 84 DAYS |
|--------------------------------------|---------|---------|---------|
| | mgm. | mgm. | mgm. |
| Untreated soils..... | 21.2 | 43.7 | 44.5 |
| 25 mgm. $\text{NO}_3\text{-N}$ | 24.0 | 44.6 | 47.3 |
| 25 mgm. $\text{NH}_4\text{-N}$ | 26.6 | 46.3 | 46.9 |
| 50 mgm. $\text{NO}_3\text{-N}$ | 24.5 | 49.3 | 50.0 |
| 50 mgm. $\text{NH}_4\text{-N}$ | 22.6 | 52.2 | 52.6 |

subtracted from the $\text{NO}_3\text{-N}$ leached from the fallow pots undergoing a similar treatment.

Although additions of the nitrogenous salts have enhanced the plant growth, yet amounts varying from 22.5 per cent to 30.4 per cent cannot be accounted for in the crop and in the leachings. This part that is not readily leached may have been assimilated by soil microorganisms which have derived their energy from the plant residues in the soil. However, plate counts showed practically no difference in numbers of organisms found in the fallow and in the planted soils.

Nitrates in soils not leached

As the soils in the previous experiment had to undergo a severe treatment every two weeks, another series of pots were conducted under more normal conditions. Five series of pots were prepared and the fertilizers were added as in the previous experiment. For each treatment, there were nine pots, three of which were left fallow and six were cropped to oats. At intervals of 28 days, two pots containing plants and one pot containing fallow soil were removed from each series for analyses. Nitrates were immediately determined on a ten-gram sample of soil, and are reported in table 5 on the basis of 1,000 gm. Total nitrogen in the entire plant is recorded in table 6.

The results obtained from the fallow soils again indicate that a proportion of the added nitrogen has been changed to some form other than $\text{NO}_3\text{-N}$ at the beginning of this experiment, and becomes slowly available, until finally there is recovered a greater amount of nitrogen than was originally added as a fertilizer. The added salts have caused some of the combined soil nitrogen to become available. A greater effect was noticeable with NaNO_3 than with $(\text{NH}_4)_2\text{SO}_4$.

That the plants did not utilize all of the $\text{NO}_3\text{-N}$ is readily noted, but the addition of the nitrogen fertilizers has increased the growth of the plant to a slight degree and greater growth is obtained with increments of nitrogen. The nitrogen recovery within the plant does not account for all of the $\text{NO}_3\text{-N}$ that has been changed.

DISCUSSION

A part of the soluble nitrogen is very rapidly changed to an insoluble form, and cannot be determined by the methods used for analysis. Plants growing upon the soil increase this "unaccounted for" nitrogen, but this increase cannot be attributed wholly to microbial activities because the numbers of microorganisms show no marked variation, and neither does the total nitrogen in the soil present marked differences. Hence another causal agent must be sought.

It is possible that the nitrate radical forms a direct chemical or physical union with the soil complexes, the plant acting in some manner as a stimulatory agent. These soil complexes, being in a colloidal form, are able to unite with the nitrate radical (2), forming compounds similar to the oxychlorides (7). As the pH of the soils used varied from 5.5 to 6.2, the conditions were favorable for such a combination.

SUMMARY

1. Fertile soils treated with NaNO_3 and $(\text{NH}_4)_2\text{SO}_4$ were kept under varying conditions, and their $\text{NO}_3\text{-N}$ content was determined.
2. Incubation of a soil increases the total $\text{NO}_3\text{-N}$ content. This is not a continuous increase but is either more or less than the amount of nitrogen added as a fertilizer.

3. A portion of the nitrogen added in the salt is rapidly changed to some form that is not readily leached.
4. This insoluble nitrogen becomes available at later periods, as is indicated by the leachings from the soil.
5. The nitrogen in the crop and in the leachings is less than the total nitrogen originally available. Of the available nitrogen, 22.5 to 30.4 per cent is retained in the soil.
6. In the soils not undergoing leaching, a similar retention of the nitrogen occurs.
7. The addition of NaNO_3 and $(\text{NH}_4)_2\text{SO}_4$ mineralizes a part of the soil nitrogen.
8. Even on fertile soils, additions of NaNO_3 and of $(\text{NH}_4)_2\text{SO}_4$ influence plant growth.
9. The plant has a depressive influence upon the accumulation of nitrates in the soil.
10. Lysimeters having 1,000-gm. capacity are described.
11. A suggestion is presented to explain the fate of the added nitrogen that has not been recovered in the plant or in the leachings.

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DIURNAL, AVERAGE, AND SEASONAL SOIL TEMPERATURE CHANGES AT DAVIS, CALIFORNIA

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During the fall and spring months there appears to be more interest than at other seasons of the year in the fluctuations of air temperature, probably because they may be of sufficient magnitude to damage crops. Maximum and minimum air temperatures, if of sufficient duration, are of considerable importance in their relation to crop development. The mean daily, monthly, and annual air temperatures as given in most reports are usually obtained by averaging the highest and lowest temperatures. Monthly means are the temperatures usually given in climatological tables, but during the past few years a shorter time, such as a week or a 10-day period, has been used occasionally.

Shaw (6) states that, "In so far as the application of meteorological data to agriculture or phenology is concerned, the week is a much more convenient unit of time than the month." The weekly record prepared by the British Meteorological Office is in the form of accumulated temperatures above 42°F. Geiger (4) has shown that the climate of the atmosphere near the soil is of quite different character from that at higher elevations. He clearly shows that the nature of the ground cover is as important a consideration as the topography and other factors. Plants which do not extend any considerable distance above the soil are under different climatic environment than those which grow to greater heights.

Studies of the leaf temperatures of cotton by Eaton (3) indicate that at Sacaton, Arizona, the yields of certain varieties of cotton, such as the Acala, are greater during cool years than during the years with average maximum summer temperatures above 100°F. Another variety, Pima Egyptian, showed less fluctuation in yields and no relationship to the mean maximum temperatures of the summer. Eaton states that, "It appears probable that the differences in the yields of Acala upland cotton in the years of higher and lower summer temperatures as compared to the yields of Pima Egyptian cotton are associated with the differences found in leaf temperatures of the two cottons."

Patton (5) believes that the maximum air temperature of the day is more

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representative than any other present measure when it is desired to determine for the growing season a temperature that is operative in a beneficial or limiting sense. Smith (8) states that, "For the sake of convenience, the months when the mean daily temperature is between 49° and 72° are considered periods of growth for most crops."

The effective temperature is considered to be the difference between the prevailing temperature and a "zero" temperature. This "zero" temperature as stated by Smith is that temperature below which a plant will make no growth.

From the preceding it is evident that some authorities place stress on the maximum or minimum temperatures whereas others believe that the mean temperatures are of more importance.

PLAN OF EXPERIMENT

Air and soil temperatures have been obtained on the deep recent alluvial soils of the Yolo Series at Davis, California, through a period of years. Details of the experiment have been discussed by the author in another paper (7). The plot in which the soil temperatures which are reported herein were obtained has been kept free of any crop since 1923. After the first of May in general there is little rainfall until the winter rainy period commences, which is usually in October. The soil moisture variations throughout the season were naturally greatest in the surface 4 inches. The air temperatures were obtained by means of a thermograph installed in the usual U. S. Weather Bureau type of shelter. Soil temperatures were obtained at depths of $\frac{1}{2}$, 3, 6, 12, 24, and 36 inches by means of electrical resistance thermometers. The temperature from each individual thermometer was automatically recorded every 15 minutes, day and night, in degrees Fahrenheit. The temperature changes occurring each day during certain months in 1925 and 1927 and the factors influencing them have been shown in a previous paper (7).

It is the main purpose of this discussion to consider average temperatures only.

AVERAGE OR MEAN TEMPERATURES

From the mass of data collected, the daily average temperature was obtained: first, a day average, which is the average of all 15-minute readings for a particular thermometer between sunrise and sunset; next, the night average, or the average of all readings between sunset and sunrise. The day average was then multiplied by the number of hours between sunrise and sunset, and the night average multiplied by the number of hours between sunset and sunrise. The sum of these, divided by 24, gives the daily average temperature. It is believed that a more complete interpretation of temperatures and their effects is possible when the temperature condition existing during the daylight hours when plants are manufacturing food materials is segregated from the night periods. This phase of the study will be discussed in another paper.

From the daily averages, the weekly averages were calculated. The weekly averages form the basis of this paper.

TABLE 1
Weekly average temperatures for 1925 period

| DATE | AIR | TEMPERATURES AT DIFFERENT DEPTHS OF SOIL | | | | | |
|------------|-----|--|-------|-------|--------|--------|--------|
| | | ½ in. | 3 in. | 6 in. | 12 in. | 24 in. | 36 in. |
| | °F. | °F. | °F. | °F. | °F. | °F. | °F. |
| 2/27 | 50 | 51 | 49 | 49 | 50 | 51 | 51 |
| 3/6 | 54 | 60 | 55 | 54 | 53 | 53 | 53 |
| 3/13 | 44 | 53 | 51 | 50 | 50 | 53 | 54 |
| 3/20 | 53 | 63 | 54 | 52 | 52 | 53 | 53 |
| 3/27 | 61 | 70 | 62 | 59 | 58 | 56 | 55 |
| 4/3 | 49 | 54 | 54 | 54 | 56 | 57 | 58 |
| 4/10 | 53 | 63 | 61 | 58 | 58 | 58 | 58 |
| 4/17 | 61 | 72 | 68 | 65 | 63 | 62 | 61 |
| 4/24 | 52 | 62 | 62 | 60 | 61 | 62 | 62 |
| 5/1 | 59 | 76 | 70 | 68 | 67 | 64 | 63 |
| 5/8 | 64 | 78 | 76 | 74 | 72 | 70 | 67 |
| 5/15 | 57 | 69 | 68 | 68 | 70 | 69 | 69 |
| 5/22 | 51 | 70 | 70 | 68 | 67 | 68 | 67 |
| 5/29 | 66 | 80 | 76 | 75 | 72 | 69 | 68 |
| 6/5 | 60 | 72 | 71 | 70 | 71 | 70 | 70 |
| 6/12 | 67 | 83 | 78 | 77 | 75 | 73 | 71 |
| 6/19 | 71 | 87 | 85 | 82 | 79 | 76 | 73 |
| 6/26 | 80 | 95 | 88 | 87 | 83 | 78 | 76 |
| 7/3 | 74 | 90 | 87 | 87 | 84 | 82 | 80 |
| 7/10 | 73 | 89 | 85 | 85 | 83 | 82 | 80 |
| 7/17 | 80 | 92 | 87 | 88 | 84 | 82 | 80 |
| 7/24 | 77 | 92 | 88 | 89 | 87 | 85 | 82 |
| 7/31 | 74 | 90 | 86 | 87 | 84 | 83 | 82 |
| 8/7 | 74 | 91 | 87 | 89 | 86 | 84 | 84 |
| 8/14 | 70 | 85 | 84 | 86 | 83 | 84 | 83 |
| 8/21 | 69 | 84 | 81 | 84 | 82 | 82 | 82 |
| 8/28 | 70 | 84 | 81 | 83 | 81 | 81 | 81 |
| 9/4 | 67 | 82 | 81 | 83 | 81 | 81 | 81 |
| 9/11 | 63 | 79 | 79 | 81 | 79 | 80 | 80 |
| 9/18 | 63 | 76 | 75 | 79 | 77 | 78 | 78 |
| 9/25 | 65 | 76 | 74 | 77 | 75 | 76 | 77 |
| 9/30 | 60 | 74 | 73 | 77 | 74 | 76 | 77 |
| Average... | 63 | 76 | 73 | 73 | 72 | 71 | 71 |

RESULTS OBTAINED IN 1925

The 1925 period extended from February 20 to September 30, the time of sunrise varying from 4:39 during the middle of June, to 6:01 on September 30.² The time of sunset ranged from 5:49 on February 20, to 7:36 during the

² The data for sunrise and sunset were obtained from N. R. Taylor, in charge of the Sacramento, California, office of the U. S. Weather Bureau.

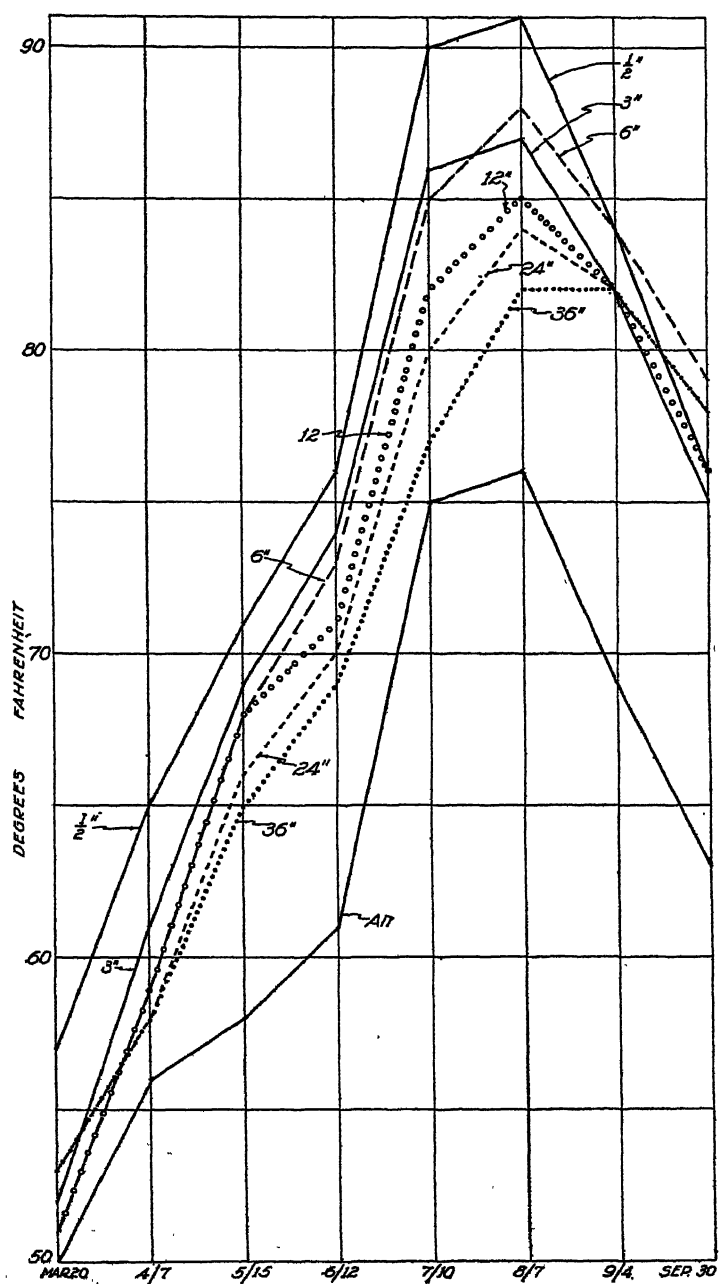


FIG. 1. AVERAGE TEMPERATURES BY 28-DAY PERIODS FOR 1925

middle of June. The length of the day was from approximately 12 to 15 hours and the night from 9 to 12 hours. The weekly averages obtained in 1925 are given in table 1, each date mentioned being for the week ending on that date.

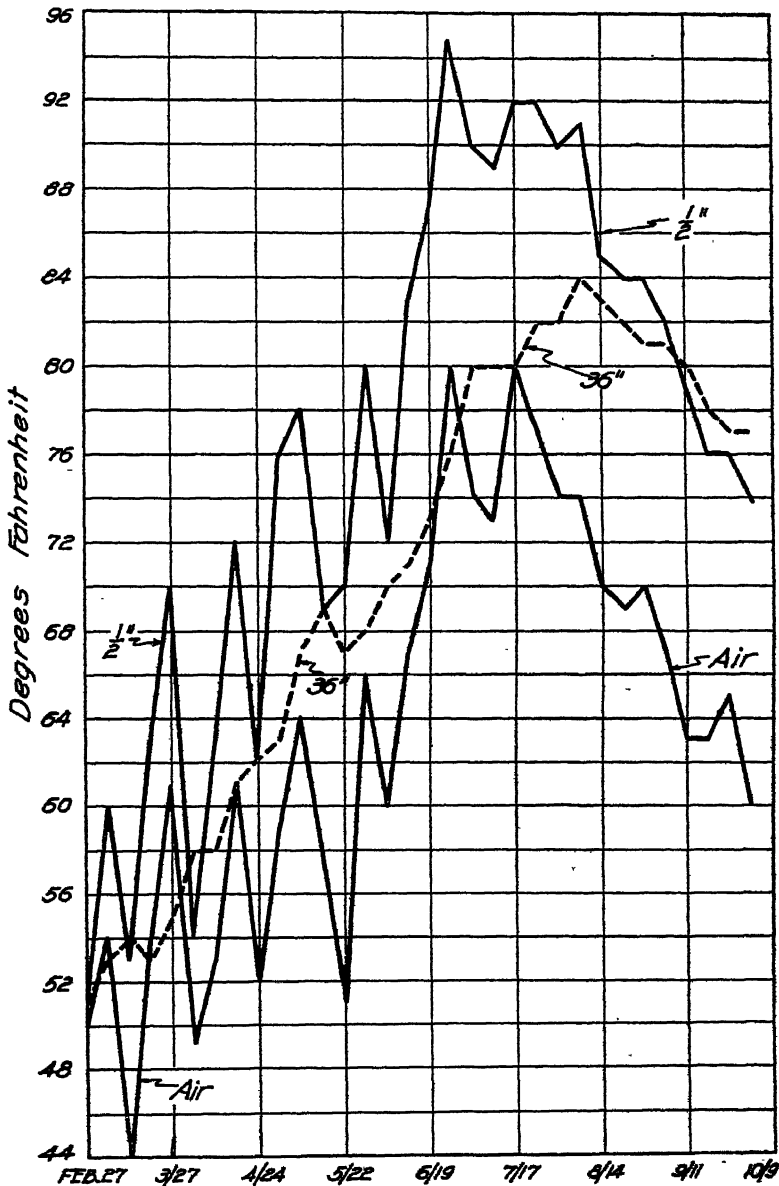


FIG. 2. WEEKLY AVERAGE TEMPERATURES FOR THE AIR, $\frac{1}{2}$ - AND 36-INCH DEPTH SOIL TEMPERATURES FOR 1925

The last period, 9/25 to 9/30, is for only 6 days. Because of failures in operating equipment there were a few times, generally not more than 3 hours in extent, when data were not obtained. From the general appearance of the temperature curves it was possible to obtain an approximate average for the day or night period. Air temperatures were obtained at a height of $4\frac{1}{2}$ feet above the soil surface.

An average weekly air temperature of 80° first occurred in the week ending June 26, as shown in table 1. All of the average soil temperatures during this week were from 2 to 8 degrees higher than during the preceding week. During the next two weeks following June 26, the average weekly soil temperatures at the $\frac{1}{2}$ -, 3-, and 6-inch depths dropped from 2 to 6 degrees, while at the 24- and 36-inch depths they increased 4 degrees. Average air temperatures of 80° were again obtained in the week ending July 17, when the average weekly temperature at the $\frac{1}{2}$ -inch depth was 3 degrees above that of the preceding week. The temperatures at the 3-, 6-, 12-, 24-, and 36-inch depths were from 1 degree to 3 degrees higher during the following week. The average temperature for the entire period for the air was 63° , while the soil temperatures ranged from 76 to 71° . The ranges in the average weekly temperatures were as follows: air, 30° ; $\frac{1}{2}$ -inch, 43° ; 3-inch, 39° ; 6-inch, 40° ; 12-inch, 37° ; 24-inch, 34° ; 36-inch, 33° .

Figure 1 summarizes the 1925 data more briefly. It gives the average temperatures by 28-day periods, the first 28-day period ending on March 20. In figure 2 only the weekly average temperatures for the air, $\frac{1}{2}$ - and 36-inch depth soil temperatures are shown, as the temperatures for the other soil depths lie, in general, between the $\frac{1}{2}$ - and 36-inch depths. In this figure the general parallelism between the air temperature and the soil temperature at the $\frac{1}{2}$ -inch depth is well shown, while the curve for the 36-inch depth is much smoother, as would be expected.

In a growing season of 140 days, extending from February 20 to July 10, the average air temperature was at this time 60° , whereas for the $\frac{1}{2}$ -inch depth, it was 72° , and for the 36-inch depth it was 64° , or a range in the soil temperatures between these two depths of 8 degrees.

RESULTS OBTAINED IN 1927

During the 1927 period, which extended from January 1 to June 18, the time of sunrise varied from 7:26 on January 1, to 4:39 on June 10 to 18. The time of sunset ranged from 4:54 on January 1, to 7:34 on June 18. The length of both day and night therefore ranged approximately from 9 to 15 hours. The weekly averages are given in table 2, each date given being for the week ending on that date.

The highest average temperatures, as shown in table 2, occurred during the last week. The greatest range in the soil temperatures occurred in the week ending April 30, when at $\frac{1}{2}$ -inch it was 77° , and at 24 inches 64° . Beginning with the week ending February 19, to the end of the week of March 12,

a range of only 2 degrees was obtained at the various soil depths. A similar period was from the week ending April 2 to the week ending April 16. The average of the air temperatures for the entire period, January 1 to June 19, was 54°, while the average soil temperatures were practically the same (61°, 60°). The range in the average weekly temperatures were as follows: air, 32°; $\frac{1}{2}$ -inch, 40°; 3-inch, 37°; 6-inch, 35°; 12-inch, 34°; 24-inch, 21°; 36-inch, 26°.

TABLE 2
Weekly average temperatures for 1927 period

| DATE | AIR | TEMPERATURES AT DIFFERENT DEPTHS OF SOIL | | | | | |
|------------|-----|--|-------|-------|--------|--------|--------|
| | | $\frac{1}{2}$ in. | 3 in. | 6 in. | 12 in. | 24 in. | 36 in. |
| | °F. | °F. | °F. | °F. | °F. | °F. | °F. |
| 1/8 | 48 | 51 | 50 | 50 | 49 | 53 | 51 |
| 1/15 | 45 | 49 | 49 | 49 | 49 | 54 | 52 |
| 1/22 | 43 | 47 | 48 | 49 | 49 | 54 | 52 |
| 1/29 | 43 | 45 | 46 | 47 | 47 | 52 | 50 |
| 2/5 | 48 | 50 | 50 | 51 | 50 | 52 | 51 |
| 2/12 | 46 | 48 | 47 | 49 | 49 | 53 | 52 |
| 2/19 | 51 | 52 | 51 | 51 | 50 | 52 | 51 |
| 2/26 | 54 | 55 | 55 | 55 | 53 | 54 | 53 |
| 3/5 | 48 | 53 | 53 | 54 | 54 | 55 | 54 |
| 3/12 | 50 | 54 | 54 | 55 | 54 | 55 | 55 |
| 3/19 | 49 | 53 | 53 | 54 | 53 | 56 | 55 |
| 3/26 | 54 | 61 | 60 | 58 | 56 | 56 | 55 |
| 4/2 | 52 | 57 | 58 | 58 | 56 | 58 | 58 |
| 4/9 | 51 | 59 | 59 | 59 | 57 | 58 | 58 |
| 4/16 | 50 | 58 | 58 | 58 | 57 | 58 | 58 |
| 4/23 | 58 | 67 | 65 | 65 | 63 | 60 | 61 |
| 4/30 | 62 | 77 | 73 | 73 | 71 | 64 | 67 |
| 5/7 | 58 | 72 | 71 | 71 | 70 | 68 | 69 |
| 5/14 | 62 | 75 | 73 | 73 | 70 | 67 | 69 |
| 5/21 | 64 | 78 | 77 | 77 | 75 | 70 | 73 |
| 5/28 | 61 | 75 | 74 | 73 | 73 | 70 | 72 |
| 6/4 | 64 | 77 | 74 | 74 | 72 | 70 | 71 |
| 6/11 | 67 | 77 | 75 | 75 | 74 | 72 | 73 |
| 6/18 | 75 | 85 | 83 | 82 | 81 | 73 | 76 |
| Average... | 54 | 61 | 61 | 61 | 60 | 60 | 60 |

It should be noted that during the last 3 weeks the average temperature at the 36-inch depth increased 5 degrees, and at the 24-inch depth 3 degrees.

In figure 3 the average temperatures by 28-day periods are shown, the first period ending on January 29. Figure 4 shows the weekly average temperatures for the air, $\frac{1}{2}$ -, and 36-inch depths. The parallelism of the air and the $\frac{1}{2}$ -inch depth temperatures is again well shown, with the 36-inch depth curve more smooth.

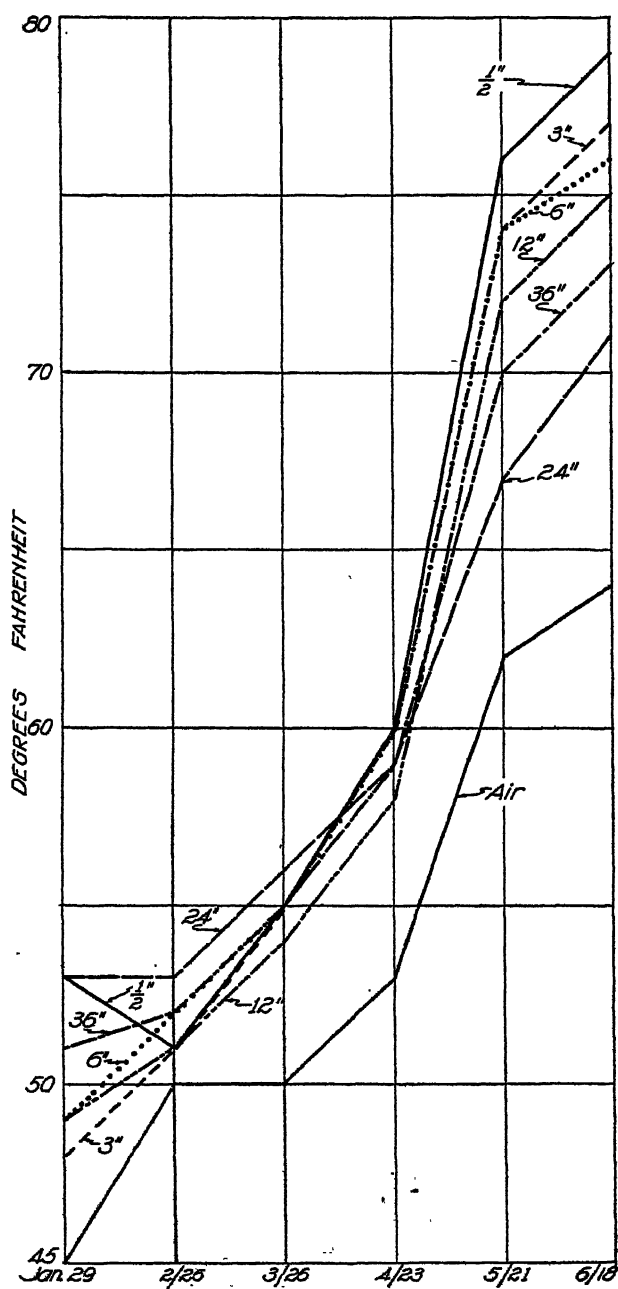


FIG. 3. AVERAGE TEMPERATURES BY 28-DAY PERIODS FOR 1927

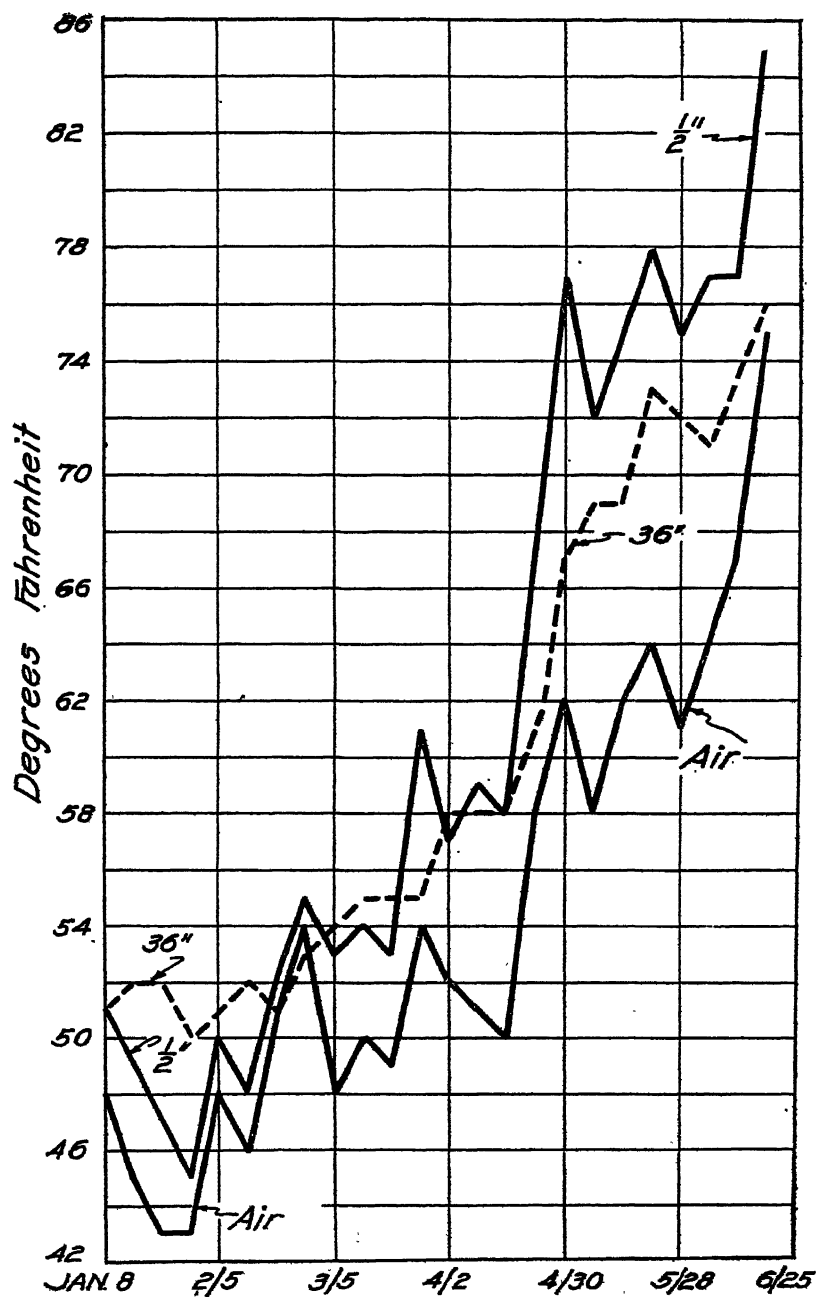


FIG. 4. WEEKLY AVERAGE TEMPERATURES FOR THE AIR, $\frac{1}{2}$ - AND 36-INCH DEPTHS SOIL TEMPERATURES FOR 1927

AIR TEMPERATURES AT MIDDAY COMPARED WITH LATE AFTERNOON

The belief that the air temperatures on summer days at 5 p.m. are higher than those at noon of the same day is borne out by the records of June, 1925, when for this 30-day period the air temperatures at 5 p.m. averaged 1.5 degree higher than those at noon. On any one day, however, the difference may be greater, or the noon readings may be the highest. On a calm, clear day the late afternoon is warmer than at midday. The character of the sky and the velocity and wind direction often change during the day and these naturally affect the comparisons. In June, 1925, on some days the 5 p.m. readings were as much as 8 degrees cooler and on other days 10 degrees warmer than the noon readings. The noon readings averaged 85.9° for the month.

METHODS OF OBTAINING DAILY TEMPERATURES

Various records have been used to express daily temperature changes. Carter (2) using standard soil thermometers has reported data for depths of 1-inch, 3-, 6-, 9-, 12-, 24-, and 36-inches occurring at Lincoln, Nebraska, by reading the thermometer once daily, at about dark. He found no apparent indication of unusually high or low air temperatures at the 24- and 36-inch depths.

Bouyoucos (1) took temperature readings at 6 a.m., 1 p.m., and 5 p.m. to obtain a daily average, as he believed that these gave "a very close approximation of the true daily average and fluctuation of temperature for all soils and for all depths." He does not claim, however, that they gave "the absolute average and range of temperature for all depths and for all soils." In order to evaluate this method, two periods were taken from the records and the daily average of the temperatures at 6 a.m., 1 p.m., and 5 p.m. were determined and compared with the averages obtained from the 96 daily readings obtained at 15-minute intervals.

Results in June, 1925

During this month the time of sunrise varied from 4:39 to 4:43 and sunset from 7:26 to 7:36. The air minimum occurred about sunrise and the air maximum from four to five hours before sunset. The lag of the maximum and minimum soil temperatures as compared to time of occurrence of the air maximum and minimum was approximately as follows: $\frac{1}{2}$ -inch—1 hour, 3-inch—2 hours, 6-inch—4 hours, and 12-inch—8 hours. Data are reported for the $\frac{1}{2}$ -, 3-, 6-, and 12-inch depths only, as it is at these depths that distinct maximums and minimums occur at Davis.

With the average of the 15-minute readings as a standard, the average obtained by the three daily readings was found to be 6 degrees higher for the air temperatures, 8 degrees higher for the $\frac{1}{2}$ -inch depth, 1 degree higher for the 3-inch depth, identical for the 6-inch depth, and 1 degree less for the 12-inch depth, for the month. Greater differences were found among individual daily readings. On some days the averages of the three daily readings were as much

as 10 degrees higher for the air temperatures, 13 degrees higher for the $\frac{1}{2}$ -inch depth, and 2 degrees higher for the 3-inch depth, than the daily (24-hour period) average of the 15-minute readings. For the 6-inch depth they were from 2 degrees less to 2 degrees more, and at the 12-inch depth they were at times 2 degrees less than the averages of the 15-minute readings.

The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three readings. The night (sunset to sunrise) averages were 31 degrees higher for the air temperatures, 34 degrees higher at the $\frac{1}{2}$ -inch depth, 6 degrees higher at the 3-inch depth, 4 degrees lower at the 6-inch depth, and 2 degrees lower at the 12-inch depth, than the corresponding average of the three readings. As the length of the day was nearly 15 hours and the night only about 9, the large differences noted between the night averages and the three readings are considerably reduced when the daily (24-hour) period is considered. The average of the three records obtained at 6 a.m., 1 p.m., and 5 p.m. during the month of June, 1925, therefore was comparable to the average of the 15-minute readings only for the 3-, 6-, and 12-inch depths and not for the air or $\frac{1}{2}$ -inch depth when the month is taken as the unit. Daily differences as noted were of greater magnitude for the air and all soil depths.

Results from May 21 to June 20, 1927

A similar comparison was made for the period of May 21 to June 20, 1927. During this time sunrise varied from 4:40 to 4:48 and sunset from 7:17 to 7:34. The occurrence of the air minimum and maximum with respect to sunrise and sunset and the lag of the soil temperatures were practically the same as during June, 1925. Again when the average of the 15-minute readings was used as a standard, the average of the three daily readings was found to be 5 degrees higher for the air temperatures, 5 degrees higher for the $\frac{1}{2}$ -inch depth, 1 degree higher for the 3-inch depth, the same for the 6-inch depth, and 1 degree lower for the 12-inch depth for the month.

As in the earlier period, greater differences were found among individual daily readings. On some days the average of the three readings were as much as 10 degrees higher for the air temperatures, 7 degrees higher for the $\frac{1}{2}$ -inch depth, 3 degrees higher for the 3-inch depth, and 2 degrees higher for the 6-inch depth than the daily (24-hour period) average of the 15-minute readings. At the 12-inch depth they were from 2 degrees less to 2 degrees more than the 15-minute averages. The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three daily readings. The night (sunset to sunrise) averages were 29 degrees higher for the air temperatures, 18 degrees higher at the $\frac{1}{2}$ -inch depth, 7 degrees higher at the 3-inch depth, from 3 degrees less to 3 degrees more at the 6-inch depth, and 4 degrees less at the 12-inch depth than the average of the three daily readings. The same conclusions relative to the value of three readings as compared to a more complete record of the temperature changes can be drawn for this (1927) period as was done in the 1925 period.

SUMMARY

During the period of February 20 to September 31, 1925, the average air temperature was 63° while the average soil temperatures for the $\frac{1}{2}$ -, 3-, 6-, 12-, 24-, and 36-inch depths ranged from 76° to 71°. The ranges in the average weekly temperatures were for the air, 30°; $\frac{1}{2}$ -inch, 43°; 3-inch, 39°; 6-inch, 40°; 12-inch, 37°; 24-inch, 34°; and 36-inch, 33°.

In the second period, January 1 to June 18, 1927, during certain weeks the average soil temperatures for the $\frac{1}{2}$ -, 3-, 6-, 12-, 24-, and 36-inch depths were within 2 degrees for all depths. The average air temperature in the 1927 period was 54°, whereas the average soil temperatures were practically the same (61°, 60°) for all depths. The ranges in the average weekly temperatures were for the air, 32°; $\frac{1}{2}$ -inch, 40°; 3-inch, 37°; 6-inch, 35°; 12-inch, 34°; 24-inch, 21°; and 36-inch, 26°. The importance of the week as a phenological unit and in the determination of effective temperatures is emphasized. The average temperatures for the air, $\frac{1}{2}$ -, and 36-inch depths are shown graphically for both the 1925 and 1927 periods. The parallelism of the air and the $\frac{1}{2}$ -inch soil depth temperatures is well shown and the data of the soil temperatures at a depth of 36 inches produce a smoother curve.

A comparison of the midday and late (5:00 p.m.) afternoon air temperatures was made to show that during a period such as a month, the differences are slight (1.5 degrees higher at noon) but that on certain days the 5 p.m. readings were as much as 8 degrees cooler and on other days 10 degrees warmer than the noon readings.

Daily average temperatures obtained by using three readings daily (6 a.m., 1 p.m., and 5 p.m.) were compared with the average obtained by using 15-minute readings, or 96 in a 24-hour period. Two warm periods were selected, one in 1925 and the other in 1927. The average of the three readings was comparable to the average of the 15-minute readings only for the 3-, 6-, and 12-inch depths and not for the air or $\frac{1}{2}$ -inch depth when the period taken was one month. Daily differences between the averages obtained in the two ways noted are of considerable magnitude. The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three readings.

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SOIL PROFILE STUDIES: II. METHODS USED IN THE PROFILE SURVEY OF NEW JERSEY SOILS

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Until recently the study of soils in the United States, England, and Western Europe was primarily limited to the surface, sub-surface, and subsoil. Such an approach to the study of soils was prompted by the agronomic point of view; the underlying motive was to discover the facts responsible for this or that behavior of the soil mass in relation to plant growth. The title, and in a large degree the content, of the valuable contribution of the eminent British soil scientist, Sir John Russell (7) "Soil Conditions and Plant Growth" typify the aforesaid. Little consideration has been given to the study of soils as such. In truth, soil studies dealt with soil material, not with soil—an independent natural body—the features and characters of which may be studied only by analyzing its anatomy as exposed in a cross-sectional, or profile, cut.

The voluminous data of the soil survey in New Jersey, and for that matter all over the United States, accompanied by the chemical and physical analysis of the soils, enriched our store of knowledge of the soil material, its geological and mineralogical origin, and its mode of formation and composition. It failed, however, to disclose the facts of the internal make-up and construction of the soil as a natural body or object; it failed entirely to follow the upward and downward movement and the translocation of the ingredients of the soil mass and their decomposition products through the soil body.

The discoveries made by the Russian scientists, discussed in a former paper (2), taught us that the soil in its anatomical make-up is composed of distinct layers, or horizons, genetically related, expressing definite chemical, physical, and biological characteristics and features. In the light of these, the forces responsible for their formation, or the soil-forming processes, become apparent. The characteristics and features of the horizons indicate the probable tendencies in the soil-forming processes and thus enable us to treat the soil in such a manner as to retain the desirable features and to maintain the conditions best suited for the ultimate aim of our studies—the productivity of the soil.

It is the study of the soil horizons in the soil profile with "no reference to other considerations," as expressed so forcibly by Marbut (6), that will help to unravel the mysteries of the "soil conditions in relation to plant growth."

*In paper I of this series, appearing in the July number of SOIL SCIENCE, the following footnote was omitted from p. 46, l. 24: "V. V. Poluinov has presented a paper on this subject, 'Contributions of Russian Scientists to Paleopedology'—Academy of Science, Leningrad, 1927, p. 1-33."

These brief considerations will suffice to introduce the subject of the profile studies of New Jersey soils. With the accumulation of the data on the physical, chemical, and perhaps biological properties of the soil material from the profiles investigated, the various relationships manifesting themselves within the soil profile will be discussed and analyzed. This, however, is to be preceded by a presentation of the methods used in the investigations. Because of the scantiness of material reported in the English literature on methods of soil profile studies—save that of Marbut's outline (6), which is excellent for a general orientation—it was deemed advisable to present the methods somewhat in detail.

METHODS

Choice of location

The accumulated knowledge on the soils of the state in the hands of the Soil Survey and the system of the Survey in arranging the soils into definite series and types¹ were utilized in determining to a certain extent the locality for the profile studies. The underlying motive in this respect was an attempt to correlate the already known facts with those sought by the comprehensive study of the profile, which is an aim by itself. The most important soil series and characteristic types within them were kept in mind, while, in general, the choice of the locality was determined primarily by the principles underlying the genetic point of view of soils.

Well-developed typical soil individuals were selected on well-drained areas, free from undulations due to the microrelief. Preference was given to virgin soils, since cultivation destroys the natural constitution of the surface horizon or horizons, thereby influencing the lower horizons and masking the nature of the effects of the soil-forming processes. Observations and data obtained on virgin soil profiles lend themselves to interpretations, which may be then applied to tilled soils of the same genetico-morphological nature. Studies on tilled soils will follow the profile survey of the virgin soils.

A preliminary study of the Soil Survey maps and reports was made as an orientation of the geography, topography, geology, and, to some extent, the climatic conditions of the area in which the profile cut or cuts were made. For future reference, the particular spot has been marked on the reference map.

Digging the soil profile cut

A trench 2.5 to 3 feet wide and as deep as required to reach into the parent material was dug for each profile. The length of the trench was determined by the depth required. Both sides and one end of the trench were cut down to form vertical walls. The other end was dug down to a slope with a step-like arrangement. This makes the trench easily accessible and affords a comfort-

¹ "Type" in the sense of a textural unit and not in the sense used by the Russian school of soil science, which indicates a certain soil-forming process, such as podzol "type" and chernozem "type."

able seat while observations and notes are being made. These steps also serve as a support for the worker when the spade is forced horizontally into the opposite end of the trench along the plane of the horizons to dig out a block of soil from any particular horizon for sampling.

In laying out the trenches to be dug, two important things were considered: First, the position of the trench was marked off on an area visibly free from heavy roots,² especially toward the vertical end of the trench. Heavy roots traversing the path of the exposed cut at that end obscure somewhat the features of the horizons and make it difficult for sampling. Second, the same end of the profile should be exposed so as to insure proper light effects (direct sunlight is to be avoided) for the differentiation of the color changes in the horizons.

The soil material dug out is thrown over to one side away from the edge of the trench; the dead leaves and wood are removed from the edges of the trench leaving the leaf-mold layer exposed.

Examination of the morphological features

The procedure followed in the examination of the soil profile was the one practiced by the leading Russian soil morphologists, with whom the authors were in contact while on the transcontinental tour with the First International Congress of Soil Science [1927],³ and described in the voluminous Russian literature, cited in a former publication (2). A number of terms used by soil workers in the English-speaking countries in designating certain characteristics of soil material have been substituted and included in the descriptions used in the procedure. Thus, for instance, the excellent term "texture" has been substituted for "soil skeleton," which is used by the Russians.

The successive steps of the procedure may be presented, following the scheme of Zakharov (8), in outline form as follows:

1. Constitution: compactness and consistency of the soil.
2. Habitus of the profile.
3. Depth of profile and thickness of respective horizons.
4. Texture of soil material.
5. Color of soil.
6. Structure
7. Concretions, foreign intrusions.
8. Miscellaneous observations.

The methods of approach in studying each one of the enumerated soil profile attributes were as follows:

1. *Constitution*.—By tapping the soil with gentle downward strokes with a sharp, rounded, shallow hand scoop or similar instrument along the surface of the wall of the exposed cut, one may readily feel in his hand when the scoop

² The virgin soils in New Jersey are almost all covered with woods.

³ Proceedings and papers of the First International Congress of Soil Science, v. 1, published in 1928.

hits a more compact or a looser horizon. After a little practice the various horizons in the soil profile may thus be differentiated and outlined.

The separate horizon established, a closer examination of them is undertaken. One may note the constitutional attributes: porosity, ease of falling apart of the structural soil units when crushed lightly in the palm of the hand, stickiness, plasticity, friability, looseness. In the B horizon of accumulation there is a sharp rise in compactness, a fundamental constitutional character of this horizon, and an increase in plasticity, especially in the humid regions. The alkali soils or the heavy type of chernozem in the semi-arid regions may possess such compacted horizons, developed to a high degree; a pick-axe or crowbar must be used on them. Something on that order of compactness was observed by the authors in examining a soil profile on the alkali soils of Fresno, California. The soils in New Jersey, as a rule, show no such striking constitutional characters, even in the B horizon of the heavy clay soils. The overlying A horizon or subhorizons are usually of a loose or mellow constitution.

The constitution of the soil horizons is of prime importance from the practical standpoint. A soil with a loose constitution is more easily tilled; the constitution of the soil determines to a certain extent the moisture and air régime of the soil.

2. *Habitus of the profile.*—Having established the different horizons in the profile from the constitution of the soil, one may proceed to describe the habitus, or the general appearance of the profile.

In a virgin forest soil of the temperate climatic zone, like New Jersey, the surface, upon the removal of undecomposed dead leaves, woody material, and stems of dead plants, reveals a mat of humified material intermingled with some mineral soil material. It is usually a thin layer, varying in thickness from 1 to 5 cm. or more. In the regions with a cool climate this surface subhorizon may reach a thickness of 30 cm. or more, giving rise to the so-called "Roh-humus." Zakharov (9, p. 8), Kosovich (4, p. 2), and other Russian investigators call this subhorizon and the underlying subhorizon "the humus accumulative horizon," designated by the letter A. Genetically the soil-forming processes manifest themselves in the accumulation of the decayed organic materials and in the formation of this surface horizon.

In the grass lands, like the steppe or the prairie, this decay (humus)-accumulative horizon is not divided into the subhorizons as in the soils of the temperate climate. The entire surface A horizon blends in with the true eluvial or leached horizon, of which we shall speak presently.

The surface subhorizon of the humus-accumulative horizon in the New Jersey soil profiles studied by the authors was designated as A_0 . This horizon is accumulative in another sense: it becomes enriched with mineral substances in the mineralization process of the organic matter, which obtains these substances from the mineral fractions of the horizons below. Part of these mineral substances, as well as the mineral fraction of the intermingled soil material, undergo leaching, and in this respect A_0 is related to the horizon of eluviation.

Part of these movable mineral substances become fixed with the humus, forming the humate complexes.

The horizon immediately below A_0 possesses the humus-accumulative properties to a less degree than A_0 . It approaches in this respect the make-up of the horizon of eluviation and suffers the mechanical and chemical reactions of the same horizon. Since it differs from A_0 and is more like the horizon of eluviation, it has been designated, whenever found, as A_1 . In the heavier soils the A_1 horizon sometimes overlies the horizon of illuviation (washing in), known as horizon B. Ordinarily, however, it has a distinct eluvial horizon. In the lighter soils the A horizon—from A_0 to B—is usually divided into one, two, or more subhorizons designated as A_1 , A_2 , A_3 , etc. The constitution of the soil; its texture, structure, and color, serve as guides for subdividing the A horizon. The A_2 and A_3 subhorizons usually constitute the pronounced eluviation horizon. This horizon of eluviation is frequently called the "transitory horizon," being genetically developed between the humus-accumulative and the illuviation horizons. The chemical analyses, which are in progress in the laboratories of the experiment station, will check up the divisions made on a morphological basis. The Ca, Fe, Al, and SiO_2 contents in the soil material of the horizons give an unmistakable picture of the genetic relationships.

The B horizon of illuviation is located below the horizon of eluviation. The substances washed out from the overlying horizons by mechanical and chemical forces are in a great measure caught in this horizon. It is easily recognized by its constitution, as has been described. Often one may detect in the B horizon, on the basis of constitution, texture, concretions, and color, certain subdivisions and, in the soils studied, these were marked B_1 , B_2 , etc.

The parent material below the B horizon is designated as C. This may be rock or unconsolidated material; it is the unweathered or incompletely weathered geologic formation.

In slightly podzolized soils—a condition found in a number of soils examined in New Jersey—the general appearance of the soil profile is not fully expressed. The effects of podzolization are not morphologically apparent, although the chemical analyses unmistakably indicate podzolization.

Zakharov (9) points out that the profile characteristics of soils depend on the character of the soil-forming process. Thus the podzol type of soil formation will manifest a definite profile type and the chernozem type of soil formation, another profile type. Besides these fundamental types, which are enumerated and illustrated in the following, there may be a great variety of types due to the properties of the parent material—its texture, relief, age, and degree of development of the soil.

The fundamental types of profiles based on the types of soil-forming processes are illustrated in figure 1, reproduced from Zakharov (8). These are: 1. Podzolized type; 2. Chernozem; 3. Serozem (gray soil); 4. Alkali; 5. Bog.

3. *Depth of profile and thickness of respective horizons.* The well-drained soils examined in New Jersey vary in their depth of profile as well as in the thickness

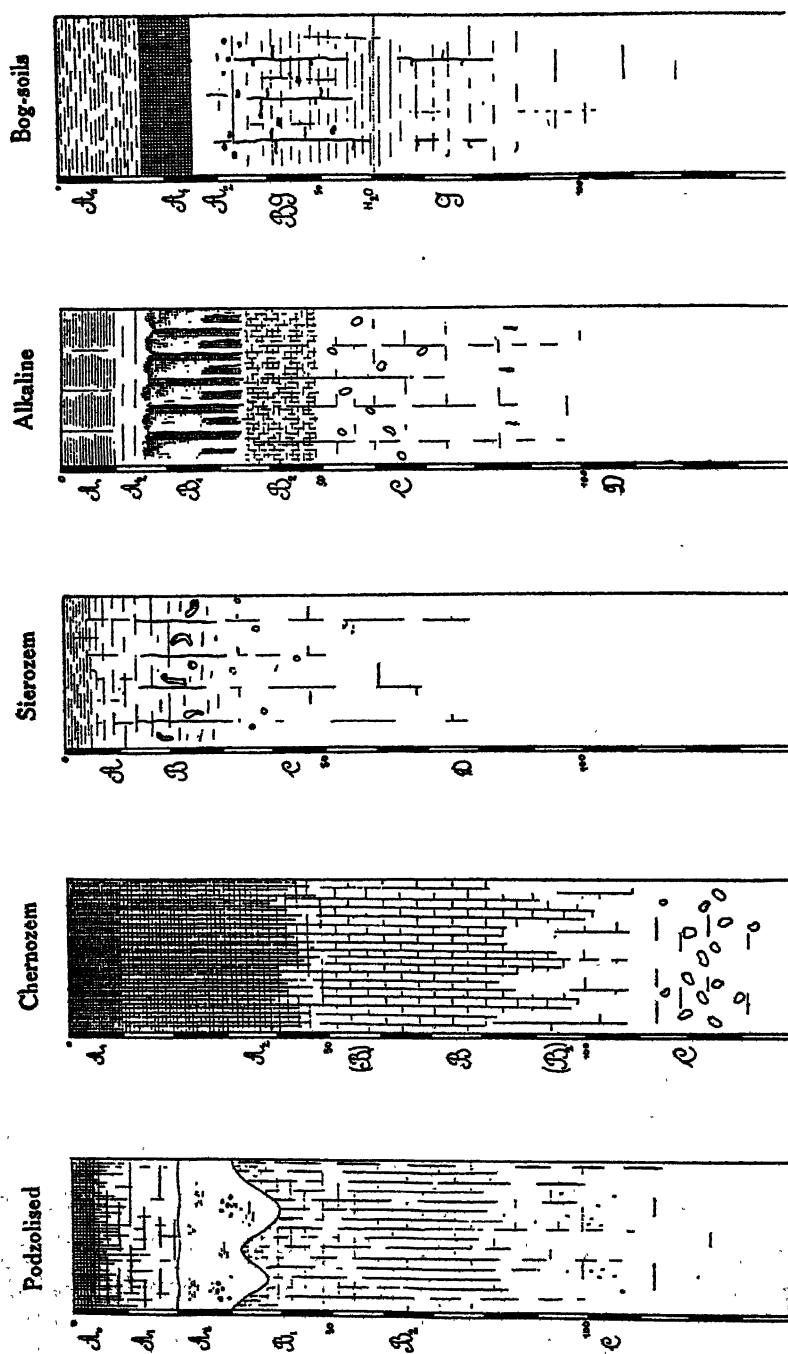


FIG. 1. PROFILE TYPES

of the individual horizons. The A_0 horizon is usually 2 to 5 cm. thick; the A (A_1 , A_2 , A_3 , etc.) horizon varies from 15 to 60 cm., with an average of 35 cm; the B horizon varies from 15 to 40 cm., with an average of 28; the total depth of the profile varies from 50 to 95 cm. (down to horizon C—the parent material). The relation (if any relation does exist) between the soil series as understood by the soil survey and depth of profile will be discussed in the forthcoming detailed studies of the profiles.

4. *Texture of soil material.*—The texture of the soil material serves as an excellent supplementary attribute in determining the constitution of the soil. In itself, the texture of the horizons is a characteristic feature of the genesis of the soil. Usually the upper horizons are of lighter texture than those below. The B horizon of accumulation, where the finer particles are washed in, is of course of the heaviest texture throughout the profile, unless the parent material is a clay.

The comprehensive scheme of the Soil Survey to classify the soil series into various types according to the texture of the surface soil has been applied in the soil profile studies to differentiate the various horizons in the profile.

The field observations on the texture are to be checked in the laboratory by the method of mechanical analyses.

5. *Color of the soil.*—The successive horizons in the profile may be differentiated according to differences in color. In some cases slight color variations may be encountered within one horizon, usually in the subhorizons of eluviation. The distribution of organic matter is usually responsible for such variations. These do not, however, impart to any horizon such properties as would differentiate it as a separate horizon. In general the color of the soil varies within wide limits, depending on the component parts of the soil—one that is rich in organic matter is dark or black in color; one rich in iron is red or yellow depending on the state of oxidation of the iron; one depleted of its organic matter and iron with a residue of SiO_2 shows a whitish-gray color.

Almost all virgin soils have a dark upper horizon, because it is the horizon of organic matter accumulation. The color of the eluvial horizon varies with the type of soil formation; thus the chernozem type is dark, the podzol is grayish-white. The general run of color in this horizon of the 11 different soil types—in the sense of the Soil Survey—investigated in New Jersey is yellowish-brown, with the exception of the Penn series which has an eluvial horizon reddish-brown to red in color. The B horizon as a rule is similar in color to that of the parent material, changing in tone because of the material washed in and incrustations formed.

The horizons of all the soils were examined for color on the freshly exposed cuts, and the soil material from each horizon was later again examined in the laboratory for color in the dry state. Usually the color of the soil material when dry attains a lighter shade.

6. *Structure of the soil.*—The type of aggregation units of soil material or the type of build of the solid portion of the soil is known as the structure. It is the

resultant of the genesis of the soil, the arrangement of the soil ingredients under the influence of the forces of soil formation. The soil structure in its natural state is a constant modified by the electrolyte content of the soil, which in turn is regulated by the weather conditions. Mechanical disturbances, like tillage and chemical treatment such as fertilizer additions, will greatly influence the structure. In recording the structure of the soil we must remember that there is a macro- and micro-structure. The micro-structure makes up the pattern of the mineralogical elements which upon cementation with the colloidal materials build the aggregation units of the macro-structure. It is the macro-structure which we can observe in the soil profile, which gives to the soil its channels, cracks, openings—in other words, its porosity. The porosity units may follow both the vertical and horizontal planes. Through these channels the gravitational waters and gases move freely; even convection currents may function through these openings. The diffusion of gases and the capillary movement of water take place within the units of the micro-structure.

The single-grain structure which indicates practically no micro-structure was noted in the sandy soils studied. In the soils of finer texture, the structure is more complicated. The method followed in designating structure was that of Zakharov (8). He recognizes three fundamental types of structure (macro): I. cubic: three equal axes at right angles to one another, II. prismatic: the vertical axis longer than the horizontal, and III. platy: the horizontal axes longer than the vertical. Within these types one may find the various forms of structure: amorphous, adobe, fluffy, cloddy, nutty, granular, crumbly, columnar, foliated, buck-shot, and different variations of these forms related to the size of the aggregate units.

7. *Concretions and foreign intrusions.*—Concretions of limestone and gypsum are very common in the soils of the less humid regions. These types of concretions are the most common; they are not, however, the only kind found. Iron and manganese concretions are frequently encountered. Depositions, replacements, and precipitation of various chemical substances along the paths of roots give what is known as veins. In some soils we have deposition of chemical substances around the structural units, giving the appearance of mycelium. The underground passages of rodents and other forms of soil-inhabiting life may also become spots of concretions. Besides these, one may find foreign material brought in by water, glaciation, and often by animals and humans. All of that is to be noted in the study of the soil profile.

8. *Miscellaneous observations.*—The depth of the water table, whenever possible, was determined and any effects on the soil due to the upward movement of the water were noted. The so-called gley formations, or mottling effects, are typical illustrations of a high water table.

In the humid belt, like New Jersey, the soils with a well-developed profile have no free carbonates in the upper horizons of accumulation and eluviation, even if the parent material is of limestone origin. On such soils the depths of the carbonate-free horizon were noted. This horizon should not be confused with the horizon of lime carbonate accumulation encountered in regions of low

rainfall: "less than 17 to 18 inches per year in cool climate and 30 inches in hot or very warm climate." [See Marbut (6)].

The depth of root penetration of the native vegetation was another point to be noted. The surface native vegetation was recorded. This is important, inasmuch as the mineral constituents derived from the organic matter influence the reaction and composition of the horizon of accumulation. The percentage of ash of the forest cover from the following species of trees as given by Glinka (1, p. 30) will illustrate the point: spruce—1.46 per cent, pine—4.52 per cent, beech—5.57 per cent, heather—3.09 per cent.

Observations were made on the distribution of organic matter in the soil profile. Such observations give an idea of the movement of the organic matter. The chemical analyses on the organic fraction in the respective horizons will show the nature of the movement and the mode of distribution of the organic matter.

Sampling of the soil profile

The next step, after the features of the soil profile have been recorded, was sampling the soil.

The respective horizons were marked off on the end and side walls of the profile cut. With the aid of a special sampling tube, described by Lebedev (5), samples were taken from the horizons on the side walls for the determination of the moisture and volume-weight of the soil. Plate 1, reproduced from Lebedev's paper, illustrates the method:

The sharp end of the steel tube (pl. 1, fig. 1) is inserted, without turning, into the wall of the soil cut at a depth of 5 to 6 cm. (pl. 1, fig. 2). With the aid of a long narrow knife the soil from the outer end of the tube is cut off at an angle so that there remains a heaping surface above the cutting edge of the tube (pl. 1, fig. 3). The tube is then removed from the soil and the surplus from the inner and outer edge is carefully removed with the sharp edge of the knife. This gives a cylinder of soil with an undisturbed structure.

The soil is placed into a tared aluminum moisture dish, weighed, dried at 100 to 105°C., and again weighed. The loss in weight gives the moisture content; the dry weight of the soil and volume of cylinder known, the volume weight of the soil is determined. Three to four samples were taken from each horizon to eliminate individual variations. Whenever pebbles were found in the sample the method of calculating the volume weight was modified, as described elsewhere (3). No samples for volume weight could be obtained by this method from horizon A₀, the so-called leaf mold, which is shallow and does not lend itself to sampling with the tube. All other horizons were sampled 2 to 3 cm. away from the upper and lower border lines of the respective horizon to insure a representative sample.

Samples of soil for physical and chemical analyses were taken in the following manner: the spade was forced into the end wall of the soil at the border line of each horizon, the upper 2 to 3 cm. of soil removed,⁴ and as much soil as neces-

⁴ In the case of the leaf mold (A) horizon the undecomposed leaves and twigs were removed before sampling.

sary taken to a depth 2 to 3 cm. above the surface of the spade. This insured a representative sample. The soil material was placed into suitable bags, labelled, brought to the laboratory, dried at room temperature, sieved through a 2-mm. sieve, and stored in glass jars ready for physical and chemical analyses. This phase of the work is now in progress, and the results will be published as they accumulate.

SUMMARY

1. A brief discussion is presented on the advantages of studying soils in their profile make-up over the old method of studying the soil material sampled at arbitrary depths.

2. The methods used in the study of the soil profile are outlined somewhat in detail and discussed.

3. The following subjects are taken up: (a) Choice of locality for making the soil cut; (b) digging the soil cut; and (c) examination of the morphological features.

4. The methods of making the observations on the morphological features and of recording them are discussed under the following headings: Constitution, Habitus of the profile, Depth of profile and thickness of respective horizons, Texture of soil material, Color of soil, Structure, Concretions and foreign intrusions, and Miscellaneous observations such as depth of root penetration and distribution of organic matter.

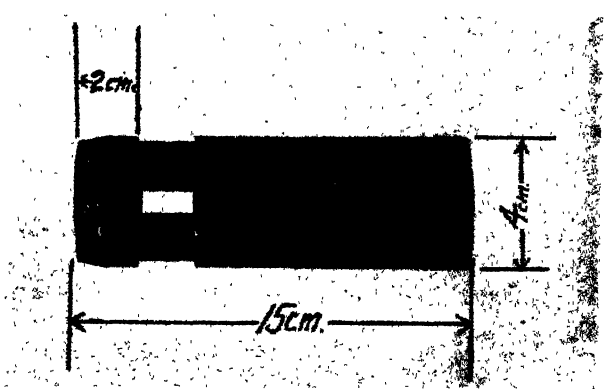
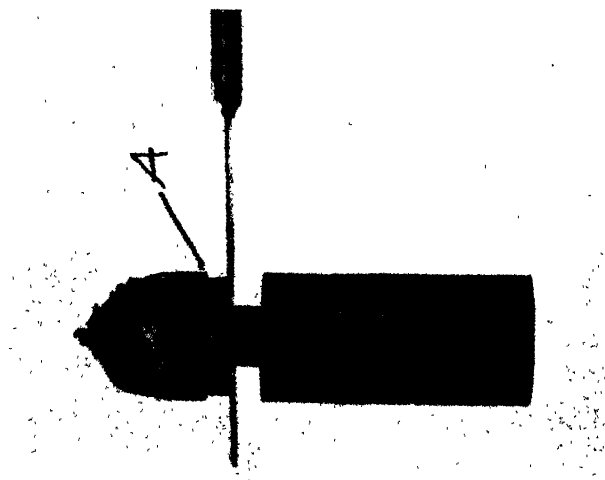
5. Methods are presented and illustrated (a) on sampling the soil for volume weight (b) on sampling the individual horizons for soil material to be used in studies on the physical and chemical aspects of the soil.

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PLATE 1

TUBE USED FOR SAMPLING SOILS FOR VOLUME-WEIGHT DETERMINATIONS



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- Coons, G. H. See Kunkel, L. O., et al.
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- Fred, E. B. See Foote, M., Peterson, W. H., and Fred, E. B.
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- Lee, L. L. See Joffe, J. S.
- Leukel, W. A., Barnette, R. M., and Hester, J. B. Composition and nitrification studies on *Crotalaria striata*, 347-371.
- Lunt, H. A. The vertical distribution of soil bases and acidity in some Illinois soils, 137-176.

SUBJECT INDEX

- Adsorption, negative, by soil colloids, 180
 Agriculture (Ackerbaulehre), a book review, 259
 Alfalfa—
 decomposition of, 15
 nodule bacteria of, 249, 441.
 Alkali soils—
 microflora of leached, 341-346
 synthetic, 341
 zinc—
 absorption of, by soils saturated with hydrogen or calcium, 417
 extraction of, by electrodialysis, 413, 416
 Ammonia, oxidation of, 131
 Ammonium—
 phosphate for rice, 88
 sulfate—
 effect on nitrate changes in fertile soil, 449
 for rice, 88
 Ammonification in alkali soils, 342
 Bacteria—*see also* Microorganisms
 nodule—
 as affected by colloid addition to medium, 444
 fermentation of glucose and xylose by, 249-256
 literature review, 436
 nitrogen fixation by, 433
 Rhizobium—
 fixation of nitrogen by, 445
 isolation of, by the use of bacteriostatic dyes, 305
 japonicum, fermentation of sugars by, 252, 255
 leguminosarum, fermentation of sugars by, 251, 254
 meliloti, fermentation of sugars by, 252, 255
 trifolii, fermentation of sugars by, 251, 254
 Bacteriological equivalents, a Dictionary of, book review, 257
 Bacteriostatic dyes, use of, in isolating Rhiz. leguminosarum, 305-313
 Barley, sewage sludge as fertilizer for, 425
 Base exchange in soil colloids, 241
 Beans, nodule bacteria of, experiments with, 440
 Beets, sewage sludge as fertilizer for, 425
 Bentonite—
 cataphoresis and flocculation of, 374
 electrodialyzed, behavior of, 207
 gel, distribution of ions in, 192
 Blood meal for rice, 88
 Botany, a book review, 259
 Braunerde, discussion on, 177
 Calcium—
 cyanamide for rice, 88
 exchangeable—
 colloid content and, 241
 method of determining, 144
 exchangeable, relation to—
 absorbed, 289
 yields, 166
 in peas, 15
 nitrate for rice, 88
 relation to nodulation of soybeans on acid and neutral soils, 261-279
 replaceable, extraction of, by electrodialysis, 413
 Carbon—
 dioxide—
 apparatus for determining, 226, 229
 method for the determination of, 225-233
 method of determining total, 225
 nitrogen ratio in plants at various growth stages, 354
 Cataphoresis, phenomenon of, in soil colloids, 373
 Cellulose—
 decomposition by organisms from alkali soils, 342
 nitrogen ratio to, in plants at various growth stages, 363
 Chemistry in Medicine, a book review, 259
 Citric acid, decomposition of, by soil, 281
 Clover, nodule bacteria of, 249, 441

- Colloidal behavior, the laws of soil, 179-220,
373-409
- Colloids—*see also* Soil colloids
activity of, 390
adsorption by, views on, 396
aggregation of, and flocculation, distinc-
tion between, 402
anion effect on swelling of, 200
as affected by—
acids, 384
bases and salts, 373
cataphoresis of soil, 373
cation, effect on composition of, 210
charge of, and flocculation, 405
charge on, present views, 396
cohesion of, 402
dehydration effects on, 205
dispersion of, 373
dissociation of, 389, 392, 394
Donnan equilibrium and, 182, 184, 376
flocculation of soil, 373
Hofmeister-ion series and, 183
hydration of, 397
ion distribution in, 192, 206
negative adsorption by, 180
plasticity, 215
potential difference in relation to viscosity
and plasticity, 217
size, as affected by—
exchange capacity, 400
ion density and dispersion, 401
size, as related to surface density of ions,
and force of cohesion, 404
valence, effect on behavior of, 185, 199
viscosity of, 213
water vapor method for determining, 237
- Corn—
sewage sludge as fertilizer for, 428
soil type and, adaptation, 109
stalks, decomposition of, 60
- Cotton seed meal for rice, 88
- Crop—
adaptation and soil type, 101-123
yields, relation of laboratory data to, 165
- Crotalaria striata, composition of, 347
- Dalea cultures, experiments with, 441
- Dentrification—
nitrite formation due to, 99
nitrogen losses by, 96
process in rice culture, 92
- Dextrins, analysis of plant, 349
- Diatase activity in plants, 221
- Donnan equilibrium and soil colloids, 182
- Electrodialysis—
extraction of adsorbed cations by, 411-421
method, 411
- Everglades, peat soils of Florida, 1-13
- Fertilizers—
Examining and Judging, Feeds, Seeds and
Soils (*Die Untersuchung und Begutach-
tung von Düngemitteln, Futtermitteln,
Saatwaren und Bodenproben*), a book
review, 259
Handbook of, a book review, 257
leunasalpeter for rice, 88
nitrogenous, availability of, to rice, 85-100
sewage sludge as, 423-432
- Field-Crop Enterprises, Including Soil
Management, a book review, 259
- Flocculation—
charge and, 405
of soil colloids, laws governing, 373
- Glucose, fermentation of, by nodule bacteria
249, 442
- Grass, sewage sludge as top dressing for, 427
- Hemicellulose, analysis of plant, 350
- Hofmeister ion series and soil colloids, 183
- Hydrometer method for determining ulti-
mate soil structure, 27
- Lignin, analysis of plant, 350
- Lime—
effect on exchangeable calcium in the
profile, 159
requirement, as affected by lime addition:
159
requirement, methods—
Hopkins, 143
Hutchinson-Maclenna, 143
- Lysimeter data on absorption versus data
obtained by the Hissink method, 294
- Magnesium—
exchangeable—
determination of, 144
extraction of, by electrodialysis, 413
relation to absorbed, 289
in peas, 15
- Microorganisms—
alkali soils, 341
number of in at different depths of peat,
322
role of, in peat formation and decomposi-
tion, 315, 326
- Microscopic Technique, Handbook of, 258
- Nitrates—*see also* Nitrogen nitrate
as affected by—
ammonium sulfate, 449

- anaerobic conditions, 92
 - sodium nitrate, 449
- formation under anaerobic conditions, 92
- leaching from soils, explanation, 198
- nitrification studies on *Crotalaria striata*, 347-371
- nitrite—
 - coccus form of, organism, 134
 - oxidation of, 131
 - oxidizing organism, isolation of, 125-136
 - rod form of, organism, 134
 - nitrites, formation of, due to denitrification, 99
- nitrogen—
 - ammonia—
 - as affected by anaerobic conditions, 94
 - in rice culture, 93
 - fixation—
 - alkali soil organisms and, 343
 - root nodule bacteria of the leguminosae and, 433-447
 - forms of extracted, in plants, 351
 - losses of, by denitrification, 96
 - nitrate—see also Nitrates
 - accumulation of, in soils, 356, 364, 450
 - in rice culture, 91
 - relation to nodule bacteria, 443
 - organic, as affected by anaerobic conditions, 95
- Nodule bacteria of leguminosae, nitrogen fixation by, 433; see also Bacteria nodule.
- Jack leaves, decomposition of, 70
- Organic—
 - matter—
 - composition and decomposition of natural, in the soil, 55-84
 - nature of decomposition, 55
 - nitrogen, as affected by anaerobic conditions, 95
- Organisms, nitrite oxidizing, method of isolation, 126
- Paleopedology or Paleoedaphology, a discussion, 46
- Pastures, Minerals in and Their Relation to Animal Nutrition, a book review, 258
- Pea, nodule bacteria of, 249
- Peat—
 - contribution to the chemical composition of, 315-340
 - microorganisms, rôle of, in formation and decomposition of, 315
 - soils of Florida everglades, 1-13
- Phosphate—
 - effect on diastase activity in soils, 221
 - Phosphoric Acid, and Phosphatic Fertilizers, a book review, 258
 - retention of, by soil colloids, 243
- Phosphorus, extraction of soil, with citric acid, 284
- Plant—
 - analysis, methods of, 348
 - influence on nitrate accumulation in soil, 453
 - juices of pea, 19
 - leaves of peas, calcium and magnesium content of, 19-23
 - material, proximate composition, 59
 - Pathology and Physiology in Relation to Man, a book review, 257
- Plants—
 - composition of, at various growth stages, 352
 - diastase activity in, 221-223
 - pea, calcium and magnesium contents of, 15-26
- Plasticity in bentonite colloid, 215
- Potassium, exchangeable, extraction of, by electrodialysis, 413
- Potato, soil type and, yields, 103
- Proteins, dissociation of, 394
- Reclamation, The Economics of Land in U. S. A., a book review, 258
- Rhizobium—see Bacteria rhizobium
- Rice, nitrogenous fertilizers availability to, 85
- Rye straw, decomposition of, 66
- Sewage—
 - analysis of, sludge, 424
 - fertilizer value of, 423
- Silica-sesquioxide ratio, relation to PO_4 retention, 245
- Silviculture—
 - Foundations of, upon an Ecological Basis, a book review, 258
 - Practice of, a book review, 258
- Sodium nitrate—
 - effect on nitrate changes in a fertile soil, 449-455
 - for rice, 88
- Soil—
 - acidity—
 - distribution of, in Illinois soils, 137-178
 - methods of measuring, discussion, 152
 - analysis methods used on some Illinois soils, 142

bases—

- calcium and magnesium, adsorbed and exchangeable, 289-304

- distribution of, in Illinois soils, 1-178

- "Braunerde," discussion, 177

- buried, discussion on, 46

- carbon-dioxide, apparatus and method for the determination of, 225-233; *see also* Carbon dioxide

- cations, extraction of adsorbed, by electro-dialysis, 411-421

- Characteristics, a book review, 257

- citric acid decomposition by, 281-287

- colloidal—

- behavior, laws of, 179-220, 373-409

- properties of, 235-247

- colloids—

- adsorption, negative, by, 180

- anion retention by, 242

- base exchange in, 241

- composition of, 238

- Donnan equilibrium, application to, 182, 184

- effect on nodule bacteria, 444

- Hofmeister ion series and, 183

- isolation of, 236

- phosphate retention by, 243

- positive and negative, 384, 387

- properties of, 236

- stability factors of, 389

- color, discussion, 475

- concretions and intrusions, discussion, 476

- cropped with oats, influence on nitrate leachings, 452

- definition of, 45

- fallow, nitrates leached from, 452

- horizons, discussion, 472

- media, phosphate in affecting diastase activity in, 221

- morphology, 39-54

- morphology features—

- color, 475

- concretions, 476

- constitution, 471

- depth of profile, 473

- habitus of profile, 472

- structure, 475

- texture of soil material, 475

- nitrates, *see* Nitrate, Nitrates

- profile—

- methods used in the, survey, 469

- sampling the, 477

- studies, 39-54, 137-178, 469-479

- types, 474

- Russian school of, science, 41

science—

- as an independent science, 39

- Handbook of (Handbuch der Bodenlehre), a book review, 260

- meetings at Danzig, 177

- slaking of, 31

- solution—

- as affected by growth of peas, 15

- calcium and magnesium in, as affected by growth of peas, 15

- structure—

- as affected by drying and wetting, 35

- discussion on, 27, 475

- synthetic alkali, 341

- temperature—

- changes at Davis, California, 457-468

- methods of obtaining, 466

- texture—

- as affected by drying and wetting, 35

- discussion on, 475

- type—

- and crop adaptation, 101-123

- effect on calcium and magnesium content of pea plants, 15

- volume-weight, 477

Soils—

- acid, relation to nodulation of soybeans, 261-279

- alkali, *see* Alkali

- buffer action of, 143, 146

- everglades peat, chemical composition, 1-13

- hydrogen-ion concentration, as affected

- by—

- citric acid treatment for P extraction, 284

- depth in the profile, 146

- growth of peas, 15

- morphological features of, 471

- neutral, relation to nodulation of soybeans, 261-279

- structure of, 27-37

Soybean—

- diastase activity of, 222

- nodule bacteria of, 249, 440

- nodulation of, in acid and neutral soils, 261

Starches, analysis of plant, 349

Sugars, analysis of plant, 349

Urea—

- decomposition of, by alkali soil organisms, 342

- for rice, 88

Viscosity of bentonite suspensions, 213

Volume weight sampling of soils, 477

Xylose, fermentation of, by nodule bacteria, 249

